Venous reserve capacity & autonomic function in formerly preeclamptic women

Ineke Krabbendam

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Chapter 1

General Introduction

Formerly preeclamptic women are at increased risk to develop hypertensive disease in their next pregnancy. Hypertensive disease in pregnancy is the leading cause of maternal morbidity and mortality in the Western World, and perinatal morbidity and mortality are also increased with this condition[1]. From these formerly preeclamptic women, those with low plasma volume seem to be at highest risk of recurrence. We studied these women in an effort to improve our understanding of the regulation of their vascular system, with the emphasis on venous and autonomic function.

Gestational hypertensive disease is a multifactorial disorder, unique to primate pregnancy. Most gestational hypertensive disease occurs in first pregnancy. Recurrent disease is associated with pre-existing hypertension, renal disease, thrombophilia, auto-immune disorders, and metabolic derangements, including the metabolic syndrome, diabetes mellitus, and hyperhomocysteinemia[2;3]. Therefore, it is appropriate to study women after their vascular-complicated pregnancy to identify any of these possible risk factors. Women with low plasma volume, mostly in conjunction with reduced venous capacitance, and higher resting sympathetic activity have been shown to be at increased risk to develop recurrent hypertensive disease in pregnancy[4-8].

Gestational hypertensive disease is caused by endothelial dysfunction late in pregnancy, but the preceding pathophysiological steps are unknown. Most studies have focused on the abnormal arterial or immunological pathway. In early pregnancy, formerly preeclamptic women with low plasma volume, as compared to women with an uncomplicated obstetric history, show a smaller increase in venous compliance and plasma volume, and a more pronounced increase in α-ANP, while arterial adaptations are unaffected[9-11]. This suggests not a disturbance in the arterial system, but a limited capacity of the venous compartment to expand. We speculate that, in women with low plasma volume who eventually develop hypertension in pregnancy, a chain of events occurs in the course of pregnancy that consists of inadequate plasma volume expansion, the inability to raise cardiac preload properly, to induce a compensatory rise in sympathetic tone, and in shear stress. This ultimately results in endothelial dysfunction in late pregnancy. It seems likely that this sequence of events is easily elicited in women with poor venous function prior to pregnancy.

The venous system contains most of the blood volume and is highly sensitive to sympathetic stimulation. Only a small increase in sympathetic activity is needed to restore a drop in venous return[12;13]. The venous compartment consists of both hemodynamically active (stressed) and inactive (unstressed) volume. Venous reserve capacity is the physiological ability to mobilize unstressed volume. A sufficient venous reserve capacity is needed to provide enough cardiac preload if circumstances demand so, such as pregnancy, physical activity, or postural change. We hypothesized that low plasma volume in formerly preeclamptic women is associated with reduced venous reserve capacity.

The aim of the thesis was to advance our understanding of the regulation of the venous and autonomic system in formerly preeclamptic women with low plasma volume as compared to women with normal plasma volume. In addition, we attempted to improve venous and autonomic function prior to pregnancy on the assumption that this might improve vascular adaptation and pregnancy outcome.

The thesis consists of 7 separate studies:

1. A literature review on venous adjustments in healthy and hypertensive pregnancy;
2. A study on the reproducibility of venous function testing during orthostatic stress;
3. A study on the reproducibility of autonomic function testing during orthostatic stress;
4. A comparative study on venous reserve capacity in formerly preeclamptic women with low and normal plasma volumes;
5. A comparative study on venous compliance and autonomic responses to orthostatic stress in formerly preeclamptic women with low, medium and high plasma volumes;
6. A comparative study on the venous storage capacity in response to volume loading in formerly preeclamptic women with low and normal plasma volumes;
7. A pilot study on the ability to improve venous and autonomic function by exercise training in formerly preeclamptic women.

Chapter 2

Venous adjustments in healthy and hypertensive pregnancy

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Marc EA Spaanderman

Expert Rev. Obstet. Gynecol. 2007; 2(5): 671-679

Abstract

Gestational hypertensive disease is generally preceded by a poor first trimester circulatory adaptation. However, the initial arterial response (i.e. drop in peripheral vascular resistance and rise in renin-angiotensin-aldosterone system) seems largely comparable. We reviewed the venous adjustments in healthy (HP) and gestational hypertensive (GH) pregnancy.

Changes in plasma volume (PV), venous compliance (VeC), α-atrial natriuretic peptide (α-ANP), inferior vena cava diameter and left atrial diameter were compared to the non-pregnant state. All showed an increase in HP. In contrast, GH pregnancy is characterized by an attenuated or even absent rise in PV and VeC with exaggerated rise in α-ANP.

We propose that a blunted venous adaptation in GH originates from an inadequate venous reserve capacity. The venous compartment is unable to accommodate the increasing PV and becomes relatively overfilled. Subsequently, α-ANP will increase, hampering further PV expansion. With increasing arterial demands in advanced pregnancy, preload can only be maintained at higher sympathetic tone. This may be the prelude to increased endothelial shear, dysfunction, ultimately leading to gestational hypertensive disease.

Introduction

Under general circumstances, 2/3 of the circulating volume is localized in the venous compartment primarily serving as a readily available buffer when arterial demands increase. Minimal changes in venous tone directly affect venous compliance and capacitance, modulating the venous return and consequently cardiac output[14]. As venous return changes atrial distension, adjustments in venous tone are accompanied by changes in α-atrial natriuretic peptide (α-ANP). α-ANP is currently the only known venous regulatory hormone controlling extra-cellular fluid volume, both interstitial and intravascular[15-17].

Healthy early pregnancy is characterized by a substantial drop in vascular resistance, thought to be initiated by a currently unknown systemically acting vasodilator[11;18-20]. Data suggest that this sudden decrease in vascular resistance is partly mediated by the opening of arterio-venous shunts. The resulting relative arterial underfill leads to several compensatory adjustments, such as elevation in heart rate and stroke volume, activation of the renin-angiotensin-aldosterone (RAA) system in absence of a rise in α-ANP, ultimately leading to plasma volume expansion[18].

The current idea on vascular complicated pregnancy is that early pregnancy circulatory responsive adaptation involves defective, a maternal reaction paralleled by shallow throphoblast invasion ultimately leading to placental dysfunction and gestational hypertensive disease[21]. The first trimester sudden drop in vascular resistance and the activation of the RAA system seems to be comparable to that observed in healthy pregnancy[9;10;22]. In contrast, the venous response seems to be blunted, as plasma volume expansion is hampered as a consequence of diminished venous compliance and upregulation of α-ANP[10;11]. Apparently, sufficient venous plasticity is at least as important as adequate arterial flexibility to meet the increased circulatory demands of pregnancy.

The aim of this paper is to review the changes of variables accounting for venous functioning in healthy pregnancy. Moreover, signs of venous maladaptation, which may be related to subsequent gestational hypertensive disease, will be considered. In these, venous functioning will be ascribed as changes in plasma volume, venous

compliance and tone, inferior vena cava diameter, left atrial diameter and alpha atrial natriuretic peptide.

Methods

Studies on plasma volume, venous compliance and tone, inferior vena cava diameter, α-atrial natriuretic peptide or factor, left atrial diameter and pregnancy were reviewed. Gestational hypertensive disease was defined as the occurrence of gestational hypertension, pre-eclampsia and/or the HELLP-syndrome (hemolysis, elevated liver enzymes, low platelets).

The percentage changes in these variables compared to pre-conceptional or (≥ 6 weeks) postpartum values, as assessed in the same study, were computed. We searched extensively to longitudinally performed studies including pre-pregnant measurements, as they reflect the most reliable data within subjects. Comparing pregnant observations to the postpartum values determined within one year postpartum may underestimate the values in pregnancy as some cardiovascular parameters may not return to baseline within one year after pregnancy[23].

The maternal cardiovascular adaptation may differ between primigravid and multigravid women[24], but many of the reviewed studies included both primigravid as multigravid women and did not perform separate analyses. Although, this might have affected the magnitude of observed responses, the direction of changes is likely to be unaltered.

As postural differences may have substantial effects on venous functioning, especially at advanced gestational age when the gravid uterus may compress the intra-abdominal venous vasculature, data were evaluated and presented accounting for posture. When present, inconsistencies or uncertainties of the reviewed studies were discussed with the author of the paper. When data were only depicted as graphs, figures were derived from the presented diagrams or plots. The results were clustered at 10 weeks' gestational age intervals and weighted based on the number of subjects participating in the study. As antihypertensive medication influences circulatory function and therefore also maternal adaptation, studies on women using

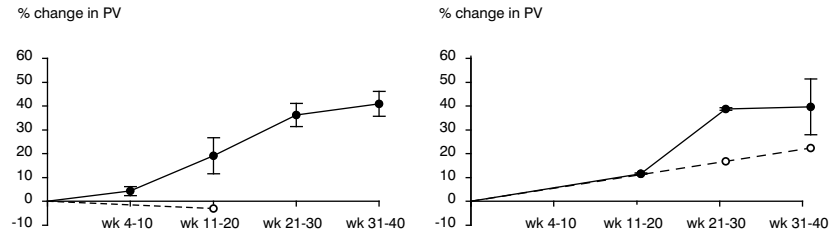
(antihypertensive) medication prior to measurement were excluded. When there were no longitudinal data on the given variable, comparisons were made on differences between healthy non-pregnant, pregnant and hypertensive pregnant women.

Venous adjustments

Plasma volume

Plasma volume responds in relation to the need to fill the vascular space and maintain blood pressure[25]. Under basal conditions 2/3 of the plasma volume is localized in the venous compartment[26]. About 40 percent of the total venous volume is actively contributing to the venous return (stressed volume) whereas 60% is hemodynamically inactive (unstressed volume) reflecting the venous reserve capacity[13].

Figure 1



Change in plasma volume (PV) in relation to gestational age in supine (left) and left lateral (right) position, in healthy (–●–) and hypertensive (–○–) pregnant women. Values are mean ± standard deviation of percentage change compared to non-pregnant state.

A chronic low total plasma volume primarily affects the unstressed volume and thereby lowers the venous reserve capacity. This condition is commonly seen in normotensive formerly preeclamptic women and in the early stages of chronic hypertension[27].

In early pregnancy, the rise in plasma volume may be triggered by the fall in afterload due to peripheral vasodilatation. The subsequent changes in perfusion pressure will trigger vasopressin concentrations and the RAA system activity to increase promoting sodium and fluid retention and volume restoration. Moreover, after the luteoplacental shift, the placenta acts as a low-resistance shunt, with a subsequent decrease in blood pressure and further stimulation of plasma volume expansion.

Accurate determination of plasma volume in pregnancy can be achieved by indicator dilution techniques, such as Evans Blue[28]. The measurement of plasma volume may be affected by the position of subjects when assessments are performed. In non-pregnant women, complete mixing of the dye is accomplished within 10 minutes. In (advanced) pregnant women, lateral position also provides mixing in 10 minutes, but in supine position the compression of the vena cava may hamper complete mixing of the dye within this period[28;29]. This could result into an underestimation of the plasma volume.

Combining the results of several studies, Figure 1 shows the relative change in plasma volume throughout gestation[28;30-40]. The rise in pregnancy begins as early as 5 weeks gestation and reaches its maximum in the third trimester.

No differences can be seen between the results in supine and left lateral position. Although methodologically we would have expected a lower PV estimation in supine position as compared to left lateral tilt, the data suggest the opposite. This may be due to methodological factors as no study measured in both positions.

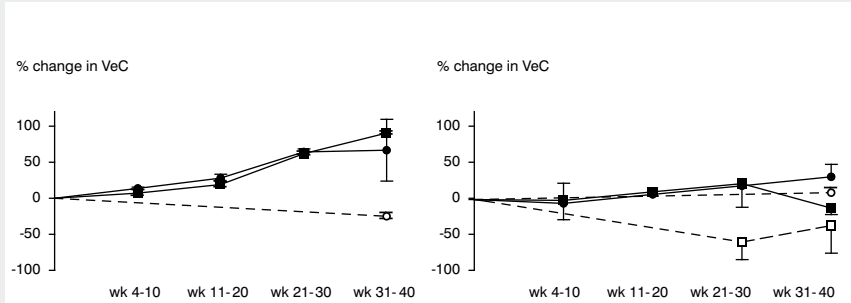
Comparing healthy and gestational hypertensive pregnancies, from our analysis, the latter group has a lower plasma volume expansion, as early as at 12 weeks gestation continuing up to the last quartile of pregnancy[30-32]. Apart from this, many studies measured the difference in plasma volume between both groups, but refrained from measuring the non-pregnant state. These studies also reported lower plasma volume in the gestational hypertensive women[41-45]. From these findings we conclude the defective plasma volume expansion starts as early as in the first trimester of pregnancy in a phase that endothelial dysfunction and pathologic capillary leakage is not present yet.

Venous compliance and venous tone

One of the most prominent features of the normal venous system is its capacity to contain volume without large increases in pressure. Venous compliance represents the accommodated amount of volume by raising pressure with 1 mmHg. In different clinical settings, like deep venous thrombosis[46;47], heart failure[48], and orthostatic intolerance[49;50], venous compliance is proven to be attenuated.

In pregnancy, both leg and forearm venous compliance can be measured by venous occlusion plethysmography. With this technique, venous outflow is hampered as arterial inflow continues. Venous compliance can be determined by the slope of the relationship between the resultant change in volume and intravenous pressure during the venous occlusion. The volume-pressure relationship is a non-linear correlation. At low venous pressures the curve is steep and therefore compliance is high, but the curve flattens with higher pressures due to lower elasticity at higher vessel diameter as well as extravasation increasing opposite tissue pressure[51]. Since in pregnancy both the ease of edema formation and vessel diameter increases, while blood pressure drops, the occluding pressure(s) used in venous

Figure 2



Change in venous compliance (VeC) in relation to gestational age in the arm (–●–) and leg (–■–) of healthy and hypertensive (–○– and –□–) pregnant women, in supine (left) and left lateral (right) position. Values are mean ± standard deviation of percentage change compared to non-pregnant state.

occlusion plethysmography may be very important in the consideration of the studies on this topic.

The reciprocal of venous tone reflects venous compliance. Therefore, we also included reports on venous tone in pregnancy in this review. In contrast, we choose to exclude studies on isolated superficial venous vessels such as on hands or fingers[52;53] or on certain venous disease states[54].

In healthy pregnancy, limb venous compliance gradually increases from first trimester on towards 60% above that observed non-pregnant by the end of the second trimester[10;55;56] (Figure 2). At left lateral tilt, forearm venous compliance is much less elevated (30%) in the last ten weeks of gestation[57-60]. When developing gestational hypertensive disease, venous compliance in the leg is approximately 40% lower throughout gestation as compared to the non-pregnant state.

The differences between both positions and between the findings in the arm versus the leg are remarkable. This might be explained by the methods employed, such as (the lack of) measuring at heart level or the used cuff pressure. In addition, edema formation in advanced pregnancy in healthy, but even more in hypertensive pregnant women, will increase tissue pressure and subsequently hamper normal volume expansion in the veins. This effect is expected to be more pronounced in the leg rather than the arm. At any rate, venous compliance rises in healthy pregnancy, while in gestational hypertensive states, venous compliance is decreased, not only as compared to healthy pregnant women, but also in relation to their non-pregnant values.

Inferior vena cava diameter

The inferior vena cava (IVC) diameter can be used as a measure of intravascular fullness[20] and is generally determined by Doppler ultrasound. With increasing gestational age, the compression of the gravid uterus affects the IVC diameter too much to obtain accurate data. To minimize this effect, it may be important to perform the measurements other than in the supine position.

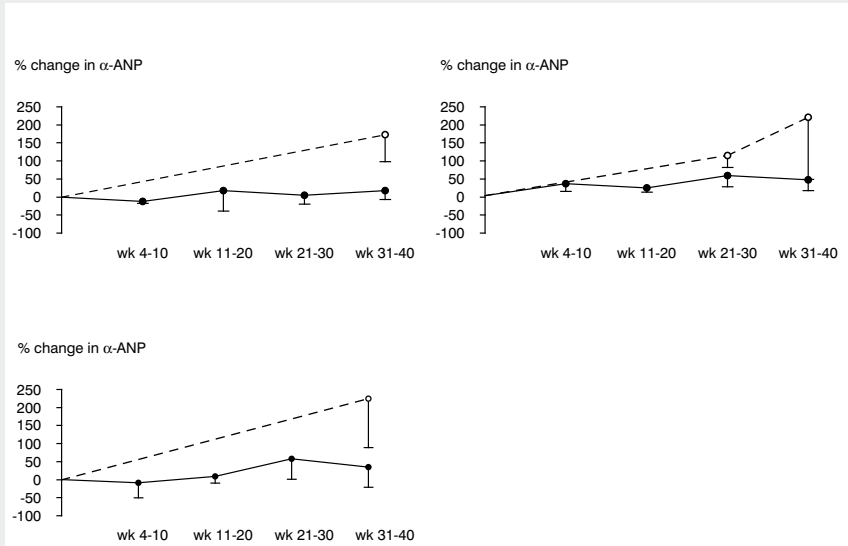
Only few studies have measured the IVC in pregnancy[61-64]. The IVC diameter increases approximately 6% during the first weeks of pregnancy[61], and continues to rise up to 70% by the end of pregnancy (in left lateral position)[63]. To our knowledge,

the change in IVC diameter in hypertensive pregnancies has never been studied. There are indications that a small IVC diameter in the second trimester is associated with the development of gestational hypertension later on in pregnancy[62;64]. In pregnancies complicated by fetal growth restriction, IVC diameter failed to increase in early pregnancy[61].

Alpha atrial natriuretic peptide

Alpha atrial natriuretic peptide is a peptide involved in volume homeostasis. It is released in response to stretch of the atrial myocytes, which can result from a rise in right atrial pressure or volume, and therefore represents the relative vascular filling state[26;65;66]. Alpha atrial natriuretic peptide is capable of causing vasodilatation,

Figure 3



Change in α-atrial natriuretic peptide (ANP) in relation to gestational age in healthy (–●–) and in hypertensive (–○–) pregnant women, in supine (upper, left), left lateral (upper, right) and sitting (lower, left) position. Values are mean ± standard deviation of percentage change compared to non-pregnant state.

natriuresis and inhibition of the renin-angiotensin system[67]. To a lesser extent, it stimulates capillary leakage. The results substantiate the physiological importance of α-ANP as a regulator of blood volume[68].

Since the discovery of this peptide by de Bold, *et al* in 1981[69], research is performed on the role of α-ANP in pregnancy. We reviewed the studies on this subject and the percentage change in α-ANP, as depicted in Figure 3[11;33;57;61;70-90].

In supine position, in healthy pregnancy, α-ANP hardly changes throughout gestation (Figure 3). When assessed in left lateral tilt, α-ANP is approximately 40% elevated. In sitting position, α-ANP may increase, but the variation is so large, that no solid conclusions can be drawn. Nonetheless, in gestational hypertensive disease, α-ANP is substantially elevated, irrespective of posture. However, the variation amongst studies is noteworthy, probably due to design, large inter-individual variation and lack of standardization at blood sampling[91].

The majority of the studies used a control group of healthy non-pregnant women, to compare the α-ANP values measured in pregnancy. Seven studies were longitudinally performed, comparing values with postpartum (6 studies) or preconceptional (3 studies) measurements. A longitudinal study is the most reliable method, but comparing to the postpartum values may underestimate the measured values in pregnancy, as Finn, et al observed still elevated α-ANP levels even at 40-120 weeks postpartum[92]. This observation suggests that only longitudinal studies starting before pregnancy may represent the most valid levels of α-ANP throughout pregnancy. These 3 studies showed an initial decrease in early pregnancy, followed by an elevation at second and third trimester[11;71;87].

Left atrial diameter

The left atrium functions as a reservoir receiving blood from the pulmonary vein. About 80 percent of the blood passively flows into the ventricle via a pressure gradient during the rapid filling phase and the remaining 20 percent is actively transferred through atrial contraction[93].

A main determinant of left atrial size is the ventricular diastolic function, but it also reflects the volume status in the great veins. The atria are extremely compliant

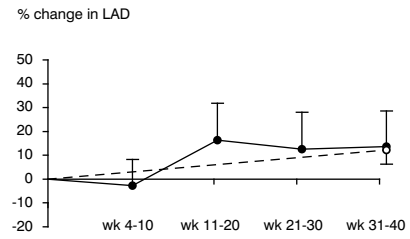
structures and respond with marked distension to increases in volume status. Therefore, in obstetric research, the left atrial diameter has frequently been used as a measure of venous filling state.

Left atrial enlargement has been proposed as a predictor of common cardiovascular outcomes, such as atrial fibrillation, stroke and heart failure[93], but has also been observed in normotensive, otherwise healthy obese people[94]. A small left atrial diameter might reflect a decreased venous filling pressure or low atrial compliance.

The atrial size can be assessed by measuring the anteroposterior diameter by M-mode echocardiography, which is a simple, but not an accurate method because the left atrium is not a symmetrical shaped structure. In contrast, left atrial volume by two- or three-dimensional echocardiography provides a more accurate estimate of left atrial size[93]. This principle is assumed not to be altered in pregnancy.

In early gestation, cardiac afterload drops. Consequently, cardiac output is forced to rise to cope with the accompanied drop in blood pressure, which in turn requires an augmented venous return. The enlarging atria with proceeding pregnancy, as

Figure 4



Change in left atrial diameter (LAD) in relation to gestational age in healthy (–●–) and in hypertensive (–o–) pregnant women, in left lateral position. Values are mean ± standard deviation of percentage change compared to non-pregnant state.

reported by the reviewed studies, reflects this enlarged venous return and the increase in vascular filling in general.

In left lateral position, left atrial diameter increased up to approximately 15% in pregnancy, but the variation between studies is large[57;70;83;95-100] (Figure 4). Only two studies reported values in supine position[61;97], observing an mean change of 1%, -1%, 6% and 4% at the first 10, 20, 30 and 40 weeks gestational age, respectively.

Data on left atrial dimension during healthy and hypertensive pregnancy are somewhat conflicting. The atrial size in gestational hypertensive patients compared to non-pregnant values was measured in 3 studies. All performed measurements in left lateral position during the third trimester of pregnancy and observed a mean increase of 15%, compared to their postpartum values[100] or to non-pregnant normotensive women[70;99]. In another 4 cross-sectional studies, gestational hypertensive women had a 6% larger left atrium compared to normotensive pregnant women[100-103] suggesting a rise in left atrial filling pressure in gestational hypertensive pregnancy.

Discussion

In this paper, we reviewed the literature on the changes in the venous system in healthy pregnancy and gestational hypertensive disease states. In healthy pregnancy, we observed a 40% increase in plasma volume and a 30% increase in venous compliance, while the inferior vena cava diameter rose up to 70% above non-pregnant values. Atrial natriuretic peptide increased about 50% in the second half of pregnancy in concert with a 15% higher left atrial diameter, reached by the end of pregnancy. The rise in venous compliance enables accommodation of the expanding plasma volume without leading directly to circulatory overfill. Nonetheless, the rise in venous compliance is unable to fully compensate for the increase in plasma volume as indicated by the enlargement of the atrial diameter and the concomitant release of α-atrial natriuretic peptide.

In women who eventually develop gestational hypertensive disease, a blunted venous adaptation occurs. A condition of relative overfill is already present at the early beginning of pregnancy, leading to atrial stretch and α-ANP release, resulting

in a diminished plasma volume expansion. In advanced pregnancy, in these women, plasma volume has only increased by 20% compared to 40% in healthy pregnant controls. The blunted rise in venous compliance and plasma volume primarily affect the physiologic pregnancy-induced increase in unstressed volume and with it venous reserve capacity[30].

These data indicate an important role of the venous system in the maternal cardiovascular adaptation. In order to create an adequate blood supply for the growing fetus, the development of a substantial amount of unstressed volume is necessary to meet the uterine demands of advanced pregnancy.

In contrast, in women who eventually develop gestational hypertensive disease, the diminished increased stressed volume can only be counterbalanced at the expense of a elevated sympathetic tone to ensure adequate venous return and with it cardiac output. The subsequent low-volume, high-output circulation will put extra mechanical shear stress upon the endothelium. Ultimately, this sets in the sequence toward endothelial dysfunction, vascular damage, poor uterine perfusion and eventually vascular gestational disease[5-7;104].

The occurrence of hypertension in pregnancy might be considered as part of a complex of (latent) cardiovascular disorders. A reduced venous compliance and capacity has been observed in studies on chronic hypertension and heart failure[14;27;48;93;105-110] but its position in the sequence towards circulatory dysfunction and disease is unclear. As formerly preeclamptic women have an increased risk to develop cardiovascular disease in later life[111;112], decreased venous reserve capacity may play a role in the development of these disorders.

In summary, a low plasma volume and venous compliance prior to pregnancy relates to blunted venous maternal adaptation and gestational hypertensive disease in advanced pregnancy.

Chapter 3

Venous response to orthostatic stress

3

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Abstract

Head-up tilt (HUT) induces a reduction in preload, which is thought to be restored through sympathetic venoconstriction, reducing unstressed volume (V_U) and venous compliance (VeC). In this study, we assessed venous inflow and outflow responses, and its reproducibility, and determined the relation with autonomic function during HUT.

Eight healthy non-pregnant women were subjected to 20° head-down to 60° head-up tilt (HUT), at 20° intervals. At each rotational step, we randomly assessed forearm volume–pressure curves (V-P curve; venous occlusion plethysmography) during venous inflow (VeC_{IN}) and outflow (venous emptying rate; VER_{OUT}). VeC_{IN} was defined as the ratio of the slope of the volume-time curve and the pressure-time curve, with direct intravenous pressure measurement. VER_{OUT} was determined using the derivate of a quadratic regression model, using cuff pressure. We defined V_u as the y-intercept of the V-P curve. We calculated for both methods the coefficients of reproducibility (CR) and variation (CV). Vascular sympathetic activity (LnLFsys) was determined by spectral analysis. VeC_{IN} decreased at each rotational step, as compared to supine position ($p<0.05$), whereas VER_{OUT} increased. CR of VeC_{IN} was higher in supine position than VER_{OUT}, but lower during HUT. CV varied between 19-25% (VeC_{IN}) and 12-21% (VER_{OUT}). HUT decreased V_u. The change in VeC_{IN} and VER_{OUT} correlated with the change in LnLFsys ($r=-0.36$, $p<0.01$ and $r=0.48$, $p<0.01$).

This is the first study in which a reproducible reduction in VeC_{IN} and unstressed volume and a rise in VER_{OUT} during HUT are documented. The alterations in venous characteristics relate to changes in sympathetic activity.

Introduction

The venous system plays an important role in hemodynamic control. About 2/3 of total blood volume is captured by the venous system[26]. An alteration in its capacity has profound effects on venous return and cardiac output. As the heart cannot store blood, venous return and cardiac output must be equal at any time. With increasing blood supply to the heart, the (stretched) ventricles increase their force of contraction (Starling's law), leading to a transient larger stroke volume and therefore cardiac output[13;14;26;113].

Women with pre-pregnant low plasma volume are prone to develop vascular complications in pregnancy[8]. Those women are unable to augment stroke volume during physical activity[114] and an extra volume load cannot be accommodated in their venous compartment[7], leading to a state of relative venous overfill. These observations suggest reduced venous dimensions, and therefore a reduced venous storage capacity.

Orthostatic stress testing is a valuable tool to measure the adjustments, and the buffering capacity, of the venous compartment. Positive head-up tilt induces an initial decrease in venous return, thereby reducing cardiac preload. The decrease in venous return will normally be counterbalanced by sympathetic stimulation. The venous system is highly sensitive to sympathetic activation[12;13;115]. Sympathetic venoconstriction, reducing venous unstressed volume, but also decreasing venous compliance, will raise venous pressure and thus right atrial pressure, filling and cardiac output[12-14]. Determination of these presumed venous responses during head-up tilt require fast methods, but these have not been established.

The aim of this study was, first, to asses the response of the venous system to orthostatic stress and, second, their reproducibility. We repeatedly assessed forearm pressure-volume changes during venous inflow, which represents the elastic property of the venous wall (venous compliance) and during venous outflow, as a measure of venous emptying rate. In addition, the relation between the venous adjustments at the graded tilt test and autonomic function was calculated.

Methods

Subjects

Eight healthy non-pregnant women participated in the study after being fully informed of the risks associated with the procedures and signing informed consent. All participants were recruited by advertisement. None of them took any medication. The study was approved by the Institutional Review Board (CMO nr. 2006/111).

All participants were studied on day 5 ± 2 of the follicular phase, to prevent hormonal influence. Participants were requested not to smoke or drink alcohol after 8pm on the day preceding the measurements. The participants were tested in 4 sessions, on two successive days. Sessions were 1,5 hour after a light breakfast and 1,5 hour after a light lunch.

Experimental protocol

Subjects were positioned on a comfortable mattress on a tilt table. The forearm was positioned at heart-level to ensure adequate venous emptying during the deflation period. In addition, the arm was stabilized to minimize muscular activity. Environmental conditions were kept constant. Room temperature was kept at 26°C and distraction due to light and sound was minimalized[116].

Participants remained supine for 10 minutes, thereafter orthostatic stress was imposed by passively changing the body posture from 20 degrees head-down (-20°) to 60 degrees head-up tilt (+60°), each time with increasing steps of 20˚ at 8 minutes interval.

Measurements

At each rotational step, venous adjustments and autonomic function were assessed in steady state. Hemodynamic changes occur immediate after rotation to realize a new hemodynamic steady state. Previous experiments in our laboratory have shown that a period of less than 60 seconds is needed to reach stability after postural change. Therefore, in the present study we decided to exclude the data of the first minute after postural change. Head-down tilt was performed to test the response with maximized venous return[117]. As Zaidi, *et al*.[118] demonstrated that little additional effect is expected with increasing tilt angles beyond 60 degrees, we evaluated orthostatic stress through head-up tilt till +60 degrees.

Venous adjustments were measured by strain gauge venous occlusion plethysmography with direct intravenous pressure measurement. An intravenous catheter was inserted in an antecubital vein and connected to a pressure transducer system at atrial height. Changes in forearm volume were measured by a mercury-in-silastic strain gauge at 5 cm distal to the antecubital crease. Changes in limb volume were expressed in milliliters per deciliter of limb tissue. A venous collecting cuff was placed 5 cm proximal to the antecubital crease. The pressure cuff was connected to a rapid cuff inflator (Hokanson E20, Denmark) to ensure rapid and accurate filling and deflation of the cuff. Data signals were recorded with a computer system, at a sampling rate of 100 Hz, and stored for further analysis (MIDAC; Biomedical Engineering Department, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands). Pressure-volume curves were assessed in two different sessions of venous inflow or venous outflow. Prior to the tilt-test, randomization determined in which order the venous inflow and venous outflow measurements would be applied, at each rotational step.

INFLOW. Cuff pressure was gradually increased from 0 to 40 mmHg in 60 seconds. Changes in forearm volume and intravenous pressure were recorded. Venous compliance (VeCIN) was defined as the ratio of the slope of volume-time curve and the slope of the pressure-time curve:

$$VeC_{IN} = \frac{\Delta\, volume \,/\, \Delta\, time}{\Delta\, pressure \,/\, \Delta\, time}$$

Only the data of the linear part of this relationship were used, until the increase rate of intravenous pressure and forearm volume diminishes.

OUTFLOW. This method is a modification of the method described by Halliwill, *et al*.[119]. Cuff pressure was immediately set at 40 mmHg (instead of 60 mmHg). We have chosen for this modification as using a cuff pressure of 60 mmHg would be inappropriate for the obstetric field. Capillary leakage is increased in pregnancy. This might induce more extravasation in pregnant women, affecting Starling forces by increasing tissue pressure. The effect is intensified when using a higher cuff pressure, as diastolic blood pressure is usually lower in (non-)pregnant women. Cuff pressure was kept at this pressure for 4 minutes. Subsequently, the cuff was

deflated to 0 mmHg in 1 minute. Pressure-volume curves were generated during deflation of the cuff. The change in forearm volume was recorded and intravenous pressure was assumed to be equal to cuff pressure. Data obtained at a cuff pressure below 10 mmHg were excluded. Pressure-volume curves were compared by means of the quadratic regression model:

$$\Delta\ volume = ß_0 + (ß_1 \cdot pressure_{cuff}) + (ß_2 \cdot pressure_{cuff})^2$$

where $ß_0$, $ß_1$ and $ß_2$ are the regression parameters. Venous emptying rate (VER_{OUT}) was defined as the derivate of the pressure-volume curve:

$$VER_{OUT} = ß_1 + (2\ ß_2 \cdot pressure_{cuff})$$

To be able to compare values within and between sessions, an (arbitrary) cuff pressure of 20 mmHg was used[119].

For both methods, we considered pressure-volume regression lines with an explained variation (r^2) > 0.9 to be representative for the original data. Only those were included for further analysis.

Unstressed volume (V_u) is defined as the volume when transmural pressure is equal to zero[12;26]. We determined unstressed volume during each rotational step for both methods. During venous inflow, the volume-pressure curve was generated using a linear model of both the volume-time curve and pressure-time curve. The y-intercept of this constructed pressure-volume curve was used to determine unstressed volume. For the outflow-method, the averaged volume-pressure curve was generated using the quadratic regression model. In this model, the regression parameter $ß_0$ was defined to represent unstressed volume[119].

Autonomic function. Fluctuations in heart rate and arterial blood pressure (ABP) were measured continuously using a finger ABP-monitoring device attached to the 3rd digit of the right hand at a sampling rate of 100 Hz (Finometer, Finapres BV, The Netherlands), to determine sympathetic activity, parasympathetic activity and baroreflex sensitivity. We derived relative brachial pressure from the finger arterial pressure by the application of waveform filtering and level correction.

We quantified autonomic activity and baroreflex sensitivity by spectral analysis technique[120]. The recordings were subdivided into data segments of 100 s, overlapping for 50%, and resampled at 5.12 Hz. Each segment was then analyzed with a Fast Fourier Transformation that searches for rhythmic fluctuations in systolic blood pressure (SBP) and pulse interval (PI) with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. Subsequently, the SBP and PI powers were expressed as a function of the frequency. Vascular sympathetic activity was defined as the natural logarithm of power of the low frequency (LF) component of the variations in systolic blood pressure and the ratio of absolute LF and high frequency (HF) powers of the pulse interval was assumed to represent the cardiac autonomic balance between the sympathetic and vagal system. Baroreflex sensitivity (BRS), which provides information about the changes in heart rate (output) in response to fluctuations in SBP (input), was defined as the (low frequency) transfer gain from SBP to PI and expressed in $ms \cdot mmHg^{-1}$.

Statistics

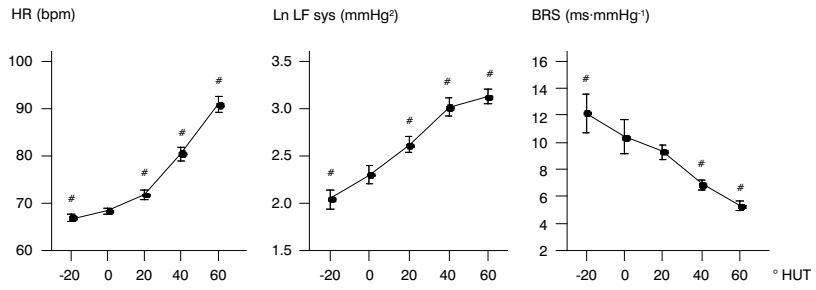
Data are presented as median (interquartile ranges), unless otherwise stated. Differences between venous responses during inflow and outflow, heart rate, sympathetic activity, cardiac autonomic balance and baroreflex sensitivity during head-up tilt were analyzed by the Wilcoxon Signed Ranks test for paired values. The pooled coefficient of variation (CV) and coefficient of reproducibility (CR) were calculated per method. CV was determined by the pooled ratio of each individual standard deviation and median value of venous compliance respectively venous emptying rate. CR at each rotational step was determined using the formula:

$$CR = 2\sqrt{2} * \sqrt{\frac{\sum (r_i - 1)\ SD_i^2}{\sum (r_i - 1)}}$$

in which r_i refers to the number of measurements in each participant (generally 4 measurements in the present study), and SD_i to each individual standard deviation[121].

Non-parametric Spearman's Rho analysis was used to test correlations between variables.

Figure 1



Heart rate (HR), vascular sympathetic activity (Ln LFsys) and baroreflex sensitivity (BRS) in response to head-up tilt (HUT). Values are presented as mean ± SEM. # $p<0.05$ compared to supine position.

Results

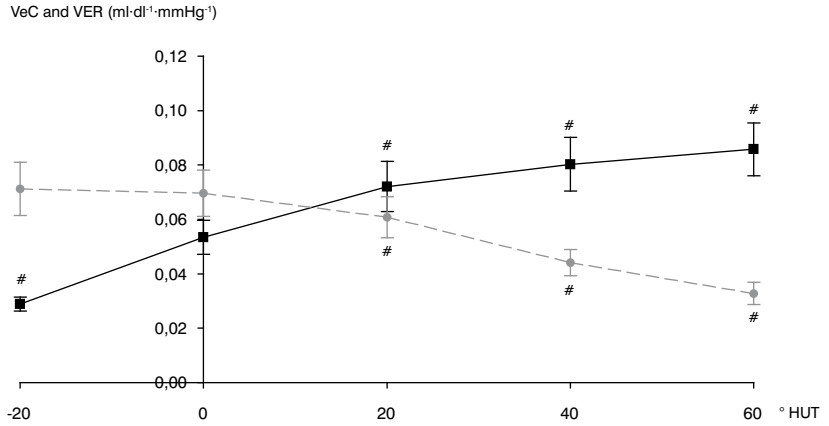
We included eight women with a median age of 25 (21-26) years and a median BMI of 23 (19-24) $kg \cdot m^{-2}$. Systolic blood pressure was 114 (110-123) mmHg and diastolic blood pressure was 60 (58-66) mmHg. None of the measurements during both venous inflow and outflow were excluded due to low r^2 of the pressure-volume regression line.

During the orthostatic stress test, heart rate and sympathetic activity increased and baroreflex sensitivity decreased (Figure 1). In supine position, VeC_{IN} was 0.066 (0.043-0.089) $ml \cdot dl^{-1} \cdot mmHg^{-1}$ and VER_{OUT} was 0.053 (0.040-0.059) $ml \cdot dl^{-1} \cdot mmHg^{-1}$ ($p<0.05$). As shown in Figure 2, VeC_{IN} decreased by respectively -35%, -45% and -53% at 20, 40 and 60 degrees head-up tilt (all $p<0.05$). In contrast, during venous outflow the effect was reversed. VER_{OUT} was +23%, +30% and +60% higher compared to supine position (all $p<0.05$). At –20 degrees head-down tilt, VER_{OUT} was 43% lower as compared to supine position, but VeC_{IN} remained unchanged.

We calculated the coefficient of reproducibility and coefficient of variation of both methods to assess reproducibility (Table 1). In supine position, the CR of the venous compliance during inflow is higher compared to the outflow (0.068 versus 0.050 $ml \cdot dl^{-1} \cdot mmHg^{-1}$), but lower during the tilt-test at 20, 40 and 60 degrees head-up tilt. The coefficient of variation of the inflow-method varied between 19% and 25% and of the outflow-method between 12% and 21%.

As sympathetic venoconstriction reduces unstressed volume to compensate for the decline in venous return, we determined the change in unstressed volume both during venous inflow and outflow. Figure 3 and 4 shows the constructed averaged volume-pressure curve, during venous inflow (using a linear model) and venous outflow (using a quadratic regression model), respectively. Using the volume-pressure curve during venous inflow, a decrease in the y-intercept during HUT was observed. In supine position, unstressed volume was 0.01 (-0.3-0.6) $ml \cdot dl^{-1}$. It decreased to -0.5 (-0.2--0.9) $ml \cdot dl^{-1}$ at +60 degrees HUT ($p=0.04$).

Figure 2



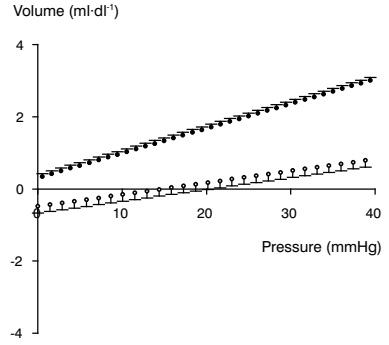
Venous compliance (VeC), measured during inflow (–●–), and venous emptying rate (VER), measured during outflow (–■–) in response to head-up tilt (HUT). Values represented as mean ± SEM. # $p<0.05$ compared to supine position.

Table 1 Reproducibility of venous compliance during head-up tilt.

Head-up tilt	VeC_{IN}	CR	CV	VER_{OUT}	CR	CV
-20 °	0.06 (0.05-0.09)	0.08	19.4	0.03 (0.03-0.03)#	0.02	13.9
0 °	0.07 (0.04-0.09)	0.07	18.8	0.05 (0.04-0.06)	0.05	12.4
20 °	0.04 (0.04-0.08)#	0.06	24.8	0.07 (0.06-0.07)#	0.07	14.3
40 °	0.04 (0.03-0.06)#	0.04	20.9	0.07 (0.06-0.09)#	0.08	20.6
60 °	0.03 (0.02-0.04)#	0.03	21.9	0.09 (0.07-0.10)#	0.08	18.6

Values represented as median (interquartile ranges). VeC_{IN}: venous compliance, measured during venous inflow, CR: coefficient of reproducibility, CV: coefficient of variation, VER_{OUT}: venous emptying rate, measured during venous outflow. VER_{OUT}, VeC_{IN} and CR presented in $ml \cdot dl^{-1} \cdot mmHg^{-1}$ and CV in %.
#p<0.05 compared to supine position.

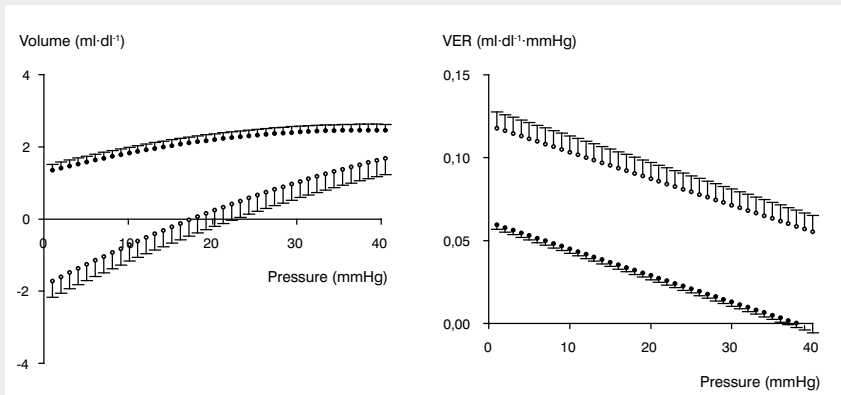
Figure 3



Model of the volume-pressure curve during venous inflow (mean and SEM), at minus 20 degrees (●) and plus 60 degrees (o) head-up tilt. Unstressed volume (y-intercept) lower at each rotational step as compared to supine.

The VER was determined using a quadratic regression model. The three parameters $ß_0$, $ß_1$ and $ß_2$ decreased with tilt (all $p<0.01$). The HUT-induced decreases in $ß_0$, $ß_1$ and $ß_2$ result in a downward shift of the volume-pressure curve and an upward shift

Figure 4



Model of the volume-pressure curve (left) and venous emptying rate (VER)-pressure curve (right) during venous outflow at minus 20 degrees (●) and plus 60 degrees (o) head-up tilt (mean and SEM). Unstressed volume (y-intercept, left curve) and venous emptying rate (at cuff pressure of 20 mmHg, right curve) are different at each rotational step as compared to supine position.

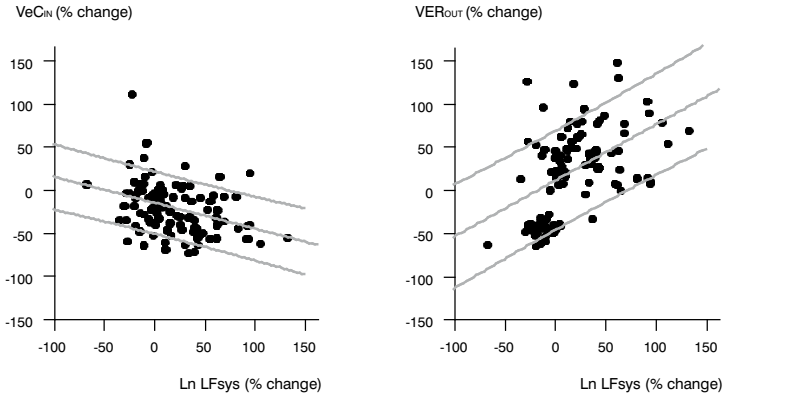
of the VER-pressure curve, which are shown in Figure 4. The parameter $ß_0$ was assumed as a representative of unstressed volume. It decreased from 1.1 (0.8-1.3) $ml \cdot dl^{-1}$ at supine position to -1.9 (-2.8- -1.3) $ml \cdot dl^{-1}$ at +60 degrees head-up tilt ($p<0.01$).

Correlation analysis showed that the change in VeC_{IN} assessed during inflow and VER_{OUT} related to the change in sympathetic activity, measured at the different tilt-angles ($r=-0.36$, $p<0.01$ and $r=0.48$, $p<0.01$, Figure 5).

Discussion

The aim of the present study was to measure the venous responses during stepwise-inflicted orthostatic stress. We assessed pressure-volume curves during inflow and outflow of the forearm veins at different tilt angles. Theoretically, head-up tilt induces an initial decrease in venous return, which is compensated by a

Figure 5



Correlation between venous compliance (VeC_{IN}, r=-0.36, p<0.01, left panel) respectively venous emptying rate (VER_{OUT}, r=+0.48, p<0.01, right panel) and the percentage change in vascular sympathetic activity (Ln LFsys).

decrease in venous capacitance through a decrease in unstressed volume and venous compliance[12-14]. To our knowledge, this is the first study in which these venous alterations, and its reproducibility, during HUT are documented.

We observed a reproducible decrease in venous compliance and unstressed volume during head-up tilt and an increase in venous emptying rate. Both observations relate, at least partly, to changes in sympathetic activity. The veins are highly sensitive to sympathetic stimulation[12;13]. Karim, et al. reported a 50% of maximum response (at 20 Hz) of the capacitance vessels at only 1 Hz sympathetic stimulation, in contrast to 10% of maximum response of the resistance vessels[115]. Sympathetic stimulation realizes an increase in stressed volume and a decrease in venous compliance[12]. Many studies observed a downward shift of the pressure-volume curve, which implicates a decrease in unstressed volume, but unaffecting the slope of the curve (and thus a similar venous compliance) during sympathetic stimulation[12;13;26;119]. We observed both a decrease in unstressed volume, during inflow and outflow, and a decrease in the slope of the pressure-volume curve, during venous inflow. In contrast to exclusively applying sympathetic stimulation by α-adrenergic agonists, cold pressure, isometric handgrip or mental arithmetic

stress as used in most studies[49;119;122-125], during HUT both an increase in sympathetic activity and baroreflex activation and a decrease in parasympathetic activity occurs. Studies on the venous response at lower body negative pressure and carotid sinus baroreflex activation suggest sympathetic stimulation of the veins via both cardiopulmonary low-pressure and high pressure receptors[123;126-129]. We speculate that the cooperative activation of these two types of receptors during HUT might contribute to the decrease in unstressed volume and venous compliance, to counterbalance the reduction in cardiac preload during HUT. Monahan, *et al.*[130] performed venous outflow measurements at the calf and observed, in contrast to our findings, no change in response to lower-body-negative-pressure (LBNP) in women. Nonetheless, both venous capacitance and unstressed volume, as indicated by the y-intercept of the volume-pressure curve, decreased, suggesting activation of the venous contractile system. As compared to our study, there are some methodological differences. First, in the Monahan study, measurements were performed at the right calf while LBNP was applied to the left leg and pelvic region. As during this procedure, women more than men tend to pool more blood in the pelvic region[131], this procedure may have affected calf venous outflow. Second, the researchers used a cuff pressure of 60 mmHg for 8 minutes, which might be supra-diastolic in (young) women, promoting fluid extravasation and with it tissue pressure. For this reason, in the present study, a cuff pressure of 40 mmHg for 4 minutes was used. Finally, as the menstrual phase may affect plasma volume, all participants in our study were, in contrast to the Monahan study, evaluated in the mid-follicular phase.

Caution needs to be taken when interpreting the relation between spectral results and venous functioning. Autonomic function, as assessed by spectral analysis, is based on the fluctuations in systolic blood pressure and heart rate. Although arterial and venous function is coupled and act in concert with each other[132], our sympathetic data are, predominantly, a measure of the arterial and cardiac sympathetic control and cannot be translated to a measure of sympathetic activity on veins.

Dysfunction at any site of the baroreflex loop interferes with sympathetic modulation of venous function. Using a control group with impaired autonomic activation, such as spinal cord injured subjects, would have contributed to the quantification of the

role of the sympathetic system in the changes in venous compliance. Subjects with paraplegia are reported to exhibit blunted change in venous compliance during head-down tilt[133], suggesting a possible role for diminished sympathetic modulation of venous compliance. However, concomitant features in this group of patients, such as assumed venous atrophy, reductions in muscle mass, effects of inactivity and compensatory mechanisms to ascertain cardiac hemodynamics, complicates to discern the role of the sympathetic nervous system in venous function[132;134-136]. Alternatively, venous sympathetic activity can be impaired by α-adrenoceptor antagonists and ganglionic blockers[132], which might be used in future research to study the contribution of the sympathetic system to venous function during head-up tilt.

We determined autonomic function by use of spectral analysis, which is a non-invasive, well validated technique and frequently used during HUT[120;137-139]. Although this method has limitations[140;141], in this study we used the changes in sympathetic activity to correlate with the venous responses. It has been reported that the changes in blood pressure variability results correspond well with the changes in muscle sympathetic nerve activity[142]. In addition, it has good reproducibility both in resting supine position and at higher tilt angles[143-145], and therefore might be valid to present.

At the inflow-method, we used the first part of the pressure-volume curve, in which a linear relationship between intravenous pressure and forearm volume is present, resulting in one value for venous compliance. The pressure-volume relationship in the vein is dependent on tissue pressure and therefore on the used cuff pressure[119;146-148]. Using the first (linear) part of the pressure-volume relationship can adequately represent venous compliance, especially when using intravenous pressure measurement, and makes comparison between different situations possible[148].

Venous compliance is generally measured in the calf. In the present study, we choose to perform measurement at the forearm to prevent influencing factors during head-up tilt. First, activation of the calf muscles is a natural response to gravitational forces. Passive tilting, using a tilt-table with footstep, as used in this study, can minimize muscle activation as compared to the response to standing. Although calf muscle activation only minimally affects volume expansion[149],

plethysmographic measures would be less reproducible. Second, loss of (intravascular) plasma volume occurs during head-up tilt due to increased fluid filtration trough capillary walls, which is also influenced by the duration of the orthostatic stimulus[150-152]. This effect might be more pronounced in the lower part than the upper part of the body during head-up tilt. In addition, stress relaxation might play a role. Stress relaxation refers to the characteristic of the vessel wall of progressive delayed stretching in response to an increase in volume, returning pressure back to normal. This phenomenon occurs in minutes to hours[26]. Measuring venous compliance at the forearm, which is placed at heart level in a stable position will minimize muscle contraction, prevents capillary filtration and diminishes the contribution of stress relaxation. However, active distribution of venous volume, by reducing venous compliance in the non-splanchnic part of the venous system, accounts for only 25% of the total blood transfer[153] and the legs taking a larger part than the arms. In the present study, we intended to qualify this response, but one must realize that the splanchnic veins are thought to play the most important role in restoring venous return as these vessels contain most venous blood[12].

Venous compliance, measured during inflow of the vein, can be assessed in 1 minute. The short duration makes this method appropriate for measurements in which brief maneuvers are allocated, as in orthostatic stress testing. Additionally, due to its slowly increasing and finally low cuff pressure[154], it is a reliable method to measure venous compliance in those with low blood pressure, as in this study.

In conclusion, head-up tilt reproducibly decreases venous compliance and unstressed volume and increases venous emptying rate. The extent of changes in sympathetic activity relate to the magnitude of venous adjustments made during HUT.

Chapter 4

Reproducibility of stepwise inflicted head-up tilt on blood pressure and heart rate variability in young, healthy women

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Fred K Lotgering
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Submitted

Abstract

Aim. The reproducibility of the hemodynamic and autonomic response pattern to graded head-up tilt (HUT) is poorly studied. The duration needed to adjust to postural change may influence stability of heart rate (HR) and blood pressure (BP), which is thought to be mandatory to accurately perform spectral analysis. The aims of this study were (1) to determine the reproducibility of hemodynamic changes during graded HUT and (2) to assess the immediate hemodynamic changes and its influence on spectral analysis.

Methods. Nine healthy women were subjected to graded HUT, from –20° (head-down) to 60° (head-up), during 4 assessments. At each rotational step, we assessed autonomic function by spectral analysis. Spectral analysis was performed on 3 periods of 5 minutes. The variation between subjects (BS), between assessments (BA) and within subjects (WS) in HR, BP and autonomic function were assessed using the standard deviation and coefficient of variation. Instability in HR and BP was assumed when at least 2 out of 3 measurements at 5 seconds intervals were outside the 90% confidence interval, using a linear mixed model.

Results. HR, BP and autonomic function had low BA variation, in contrast to the variation BS and WS. Within 1 minute after rotation, more than half of the HR and BP measurements were stable. Autonomic function was not affected by the period of instability.

Conclusion. Graded HUT is an effective test to reproducibly assess hemodynamic and autonomic responsiveness. Spectral analysis can be assessed from data recorded directly after postural change.

Introduction

Spectral analysis on spontaneous fluctuations of beat-to-beat changes in blood pressure and heart rate provides information about autonomic cardiovascular function. This technique subdivides the variability in heart rate and blood pressure into different frequency components. Specific frequency areas represent predominantly sympathetic or mixed (para)sympathetic activity, respectively[120;155;156]. The Fourier spectral analysis has widely been used to describe (para)sympathetic activity and baroreceptor sensitivity in both health and disease, like post-myocardial infarction, diabetes, brain-damage, polyneuropathy, renal failure and after vascular complicated pregnancy[139;157-161].

Orthostatic stress testing is a valuable tool to measure the adjustments of the autonomic and cardiovascular system to the initial reduction in venous return. Sympathetic activity determined by spectral analysis has been reported to have a good reproducibility at rest and at 60 or 70° head-up tilt[137;143-145]. These situations indicate the compensation of the autonomic system to a very large reduction in preload. It is however unknown if sympathetic activity measures can also be reproducibly assessed by spectral analysis if orthostatic stress stimuli are applied by introducing more graded changes to the cardiovascular system by applying intermediary rotational steps of head-up tilt. The response pattern to graded changes in head-up tilt may yield more reliable measures of autonomic activity changes than a single stimulus. It enables the assessment of the sensitivity of the autonomic system. Testing the response pattern during graded head-up tilt can only be considered methodologically useful, especially when applied in longitudinal studies requiring serial measurements such as in pregnancy, when reproducibility is good. The testing of this concept requires the assessment of the magnitude of the variations in BP and HR variability between assessments as well as between and within subjects.

Second, to assess autonomic function from spontaneous blood pressure and heart rate variations by Fourier analysis, a relatively stable hemodynamic state is required. Reproducibility may be affected by the hemodynamic changes, which occur immediately after postural change. These changes cooperate to restore venous return and to create a new steady state, but the duration of the period when steady

state is assumed to be reached varies among studies[26;118;162-165]. In addition, the time to reach a new steady state is probably affected by the intensity of the orthostatic stimulus during head-up tilt and may influence the start of the recordings which are used for the measurement of (para)sympathetic activity after postural change. To exclude these immediate hemodynamic changes, data for spectral analysis are taken when the new "steady state" has been reached.

The aims of this study are, first, to determine the reproducibility of the pattern of the hemodynamic and autonomic response during graded head-up tilt and, second, to assess the duration of the immediate hemodynamic changes in young, healthy women to serve as a reference for formerly preeclamptic women. Finally, the influence of the time needed to reach (hemodynamic) stability on the spectral analysis results at each rotational step is determined.

Methods

Subjects

Nine healthy non-smoking female volunteers between 22 and 27 years were studied on day 5 ± 2 of the follicular phase. None were taking medication. Each participant was familiarized with the equipment and experimental protocol prior to the first assessment. The study was approved by the Institutional Review Board (CMO-number: 2005/212) and written informed consent was obtained from all the participants.

Experimental design of the study protocol

All participants were subjected to 2 sessions on 2 consecutive days (4 assessments per person in total), at least 1.5 hour after a light meal. They refrained from alcohol and caffeine for at least 10 hours prior to assessment. The study protocol started at 9.30 AM.

Head-up tilt was performed after voiding the bladder and executed under controlled environmental conditions, in a quiet and partially darkened room with an ambient temperature of 26°C[116;166]. A temperature of 26°C was chosen, as this is reported to be a comfortable temperature during bed rest[116]. Moreover, this temperature is in the neutral range without either inducing vasoconstriction or vasodilatation in the skin vasculature[166].

Subjects were positioned on a comfortable mattress on the tilt table, to minimize muscular activity. The right arm was positioned at heart level. Participants remained supine for 10 minutes, after which orthostatic stress was imposed by passively changing the body posture from 20 degrees head-down (-20°) to 60 degrees head-up tilt (+60°), in steps of 20° at 10 minutes intervals. Head-down tilt was performed to measure the response at facilitated venous return[117]. We evaluated orthostatic stress through head-up tilt till 60 degrees, as little additional effect can be expected with increasing tilt angles beyond 60 degrees[118].

Measurements

Spontaneous fluctuations in heart rate (HR) and arterial blood pressure (ABP) were measured continuously by a finger ABP-monitoring device attached to the 3rd digit of the right hand (Finometer, Finapres BV, The Netherlands), at a sampling rate of 100 Hz. From these data, variations in heart rate and blood pressure between assessments, between subjects and within subjects were determined. In addition, the variation in heart rate and blood pressure was used to determine the time needed to reach hemodynamic stability for each individual assessment.

Sympathetic activity, autonomic balance and baroreflex sensitivity were assessed by spectral analysis[120;155;156]. To determine both the variation in autonomic function at each rotational step as well as the influence of the hemodynamic changes immediately after postural change, we compared autonomic function measures in 3 time periods; 0-5 minutes (PERIOD5), 1-6 minutes (PERIOD6) and 2-7 minutes (PERIOD7) after the postural change. For this purpose the 300 s data segments of the beat-to-beat data were linearly interpolated, and resampled at 5.12 Hz to convert the unequally spaced beat-to-beat time series to a uniformly spaced time series. A Fast Fourier Transformation was performed on consecutive segments of 100 s (=512 data points) which were allowed to overlap 50%. The Fast Fourier Transformation searches for rhythmic fluctuations in systolic BP (SBP) and pulse interval with a frequency range between 0 and 2.56 Hz. To minimize the influence of very low frequencies each data segment was tapered using a cosine function. The amplitude of each fluctuation determines the power at each frequency. Subsequently, the SBP and PI powers were expressed as a function of the frequency; the low frequency component (LF: 0.04-0.15 Hz) and the high frequency component (HF: 0.15-0.4 Hz). We defined sympathetic activity (LFsys) as the power of the LF

component of the variations in SBP and assumed the ratio of LF and HF powers of the pulse interval to represent the autonomic balance between the sympathetic and vagal system. Baroreflex sensitivity, which provides information about the changes in HR (output) in response to fluctuations in SBP (input), was defined as the transfer gain from SBP to pulse interval and expressed in ms·mmHg^{-1}.

Statistical analysis

In this study, we aimed at determining the reproducibility of the pattern of hemodynamic and autonomic responses during graded head-up tilt. Before designing the study, we expected an instable pattern in the first two or three minutes and a stable pattern thereafter. First, the stable pattern was studied using a linear mixed model for each of the rotational steps, separately. The dependent variable was heart rate, systolic blood pressure, diastolic blood pressure and mean arterial blood pressure, respectively. The independent regression variable was linear time. The independent random variables were the individual intercept and the assessments (n=4, two assessments on two consecutive days). The between assessment standard deviation is presented to indicate the reproducibility of the assessment. For comparison, the within subject and the between subject standard deviation is presented.

Second, we studied deviation from the stable pattern of the heart rate in the first three minutes. For this purpose, we defined the time period of instability as the period directly after postural change until the last time (within four minutes) that two out of three consecutive observations fell beyond the 90% confidence band of the expected value of that person at that point of time. At this point, the above mentioned linear model was extended with the individual linear time regression that was treated as a random variable. This allowed more individual variation to the prediction. However, because of computational limits, the confidence bands were calculated using the residuals during the last four minutes. The estimates of the stable pattern, using this model were compared to those using the model mentioned above and we found these were very similar if not identical. Thus, the computational limits did not affect the overall estimates.

The reproducibility of the autonomic function and the differences between the 3 time periods was studied, using a similar linear mixed model for each of the rotational steps, separately. The dependent variable was sympathetic activity

(LFsys), the ratio of low and high frequency component of the fluctuations in pulse interval (autonomic balance) and the baroreflex sensitivity, respectively. The independent class variable was time period (n=3). The independent random variables were the individual intercept and assessment (n=4, two assessments on two consecutive days). The between assessment standard deviation is presented to indicate the reproducibility of the assessment. For comparison, the within subject and the between subject standard deviation is presented.

Results

We included 9 non-pregnant women with a median age of 22 (interquartile range 22-23) years and a median BMI of 21 (20-22) kg·m^{-2}. Amongst the 9 participants, 1 woman reported symptoms of imminent fainting at 60 degrees head-up tilt at 3 consecutive assessments, after which the test was ended. The data till 40 degrees head-up tilt were used. In response to orthostatic stress, HR, MAP and sympathetic activity increased, whereas BRS decreased in all participants.

First, the variation in heart rate and blood pressure is shown in Table 1, Figure 1 and 2. The variation between the 4 assessments was relative low and showed a slight increase with further head-up tilt (HR: 1.0 to 1.4 bpm, MAP: 1.1 to 3.4 mmHg). The variation between subjects and within subjects was independent of the rotational step. Trend analysis showed a consistent drift in heart rate over time at each rotational step. At minus 20 degrees head-down tilt, there was a negative drift of –0.16 bpm/minute and at 60 degrees HUT it was +0.21 bpm/minute. This resulted in a -1.12 bpm lower heart rate at the end of the -20° head-down tilt. The positive drift at 60 degrees resulted in a +1.47 bpm higher heart rate at the end of this rotational step (Figure 2). The variation in autonomic function between assessments is low at all positions, especially in supine position and at -20 degrees head-down tilt (Table 2). In contrast, the variation in LFsys between subjects and within subjects is relative high, varying between 34% and 96%. BRS showed a good reproducibility with reliable variation between assessments (range 0-8%), between subjects (16-29%) and within subjects (20-35%). Autonomic balance (LF/HF) had high variation between subjects (61-72%), but relative low variation between assessments (0-21%) and within subjects (36-52%).

Table 1 Reproducibility of venous compliance during head-up tilt.

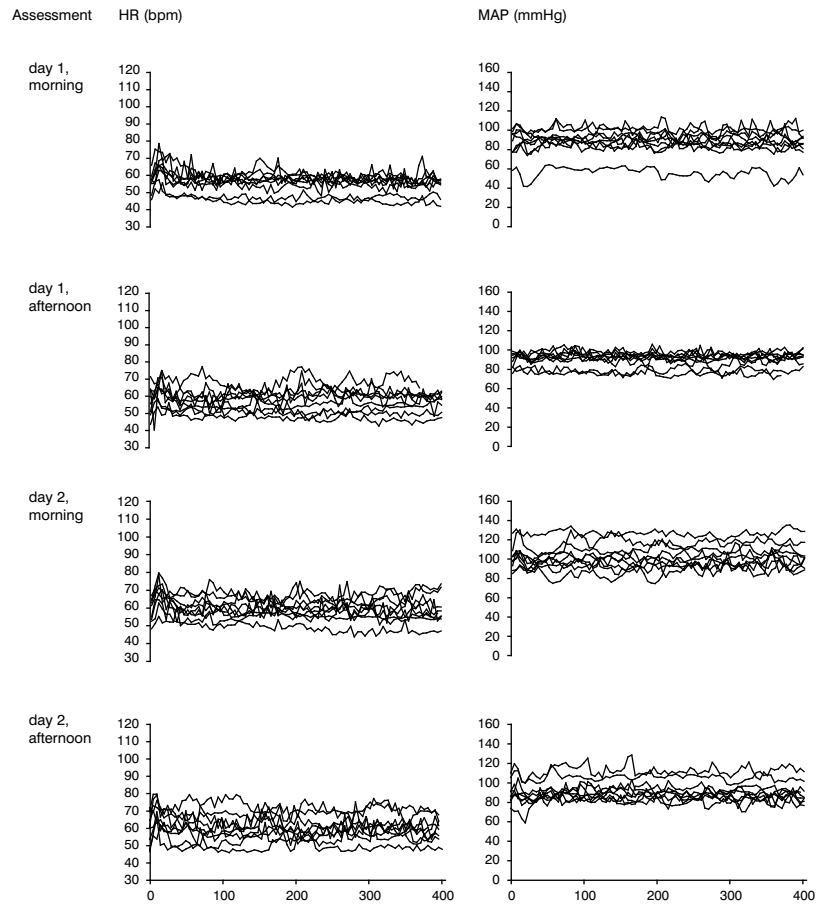
		-20		0		20		40		60	º HUT
HR	BS	5.7	(9.7%)	6.5	(11.0%)	9.4	(16.2%)	8.3	(11.8%)	11.3	(13.2%)
	BA	1.0	(1.7%)	1.7	(2.9%)	1.6	(2.8%)	1.8	(2.6 %)	1.4	(1.6 %)
	WS	4.4	(7.5%)	4.1	(6.9%)	4.1	(7.0%)	5.4	(7.7%)	6.1	(7.1%)
SBP	BS	15.2	(11.7%)	14.7	(11.3%)	23.3	(17.7%)	10.7	(8.4%)	9.1	(7.5%)
	BA	2.6	(2.0%)	2.0	(1.5%)	1.6	(1.2%)	1.7	(1.3%)	3.8	(3.1%)
	WS	7.8	(6.0%)	8.6	(6.6%)	8.8	(6.7%)	7.5	(5.9%)	8.2	(6.8%)
DBP	BS	6.2	(9.2%)	8.2	(12.1%)	7.8	(11.3%)	6.9	(9.7%)	4.5	(6.1%)
	BA	1.2	(1.8%)	1.8	(2.7%)	1.3	(1.9%)	1.8	(2.5%)	3.0	(4.1%)
	WS	6.6	(9.7%)	7.3	(10.8%)	7.2	(10.4%)	5.7	(8.0%)	5.8	(7.9%)
MAP	BS	7.8	(8.6%)	10.0	(11.0%)	10.7	(11.6%)	7.1	(7.8%)	5.0	(5.5%)
	BA	1.1	(1.2%)	1.9	(2.1%)	1.3	(1.4%)	1.9	(2.1%)	3.4	(3.8%)
	WS	7.4	(8.2%)	7.9	(8.7%)	7.7	(8.3%)	6.2	(6.8%)	6.4	(7.1%)

HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial blood pressure, BS: variation between subjects, BA: variation between assessments, WS: variation within subjects.

Second, Figure 2 shows the overall mean HR and BP (± standard error of the mean) at each 5-seconds interval. The period of instability can be recognized by a temporary rise in heart rate and a decrease in (diastolic) blood pressure. We determined the point of stability using the individual profiles of the variation in heart rate and blood pressure of each participant, at each assessment and rotational step. Figure 3 shows 2 individual heart rate profiles and their point of stability. Note that the second participant shows a (possible) second period of instability. The last point was chosen as the start of the stable period. In supine position, the point of stability in heart rate was reached within one minute in 61% of all 36 assessments and increased to 81% within 3 minutes (Figure 4). Stabilization occurred at 60 degrees HUT in 53% of assessments within one minute and in 83% within 3 minutes. At minus 20 degrees, the percentage stability was comparable to supine position. Thereafter, there was a slight decrease at each rotational step towards 60 degrees head-up tilt. Similar results were obtained on systolic and diastolic BP. Of all supine systolic BP data, 67% and 91% were within the 90% confidence interval within the first and third minute, respectively. At 60 degrees HUT, it decreased to 50% and

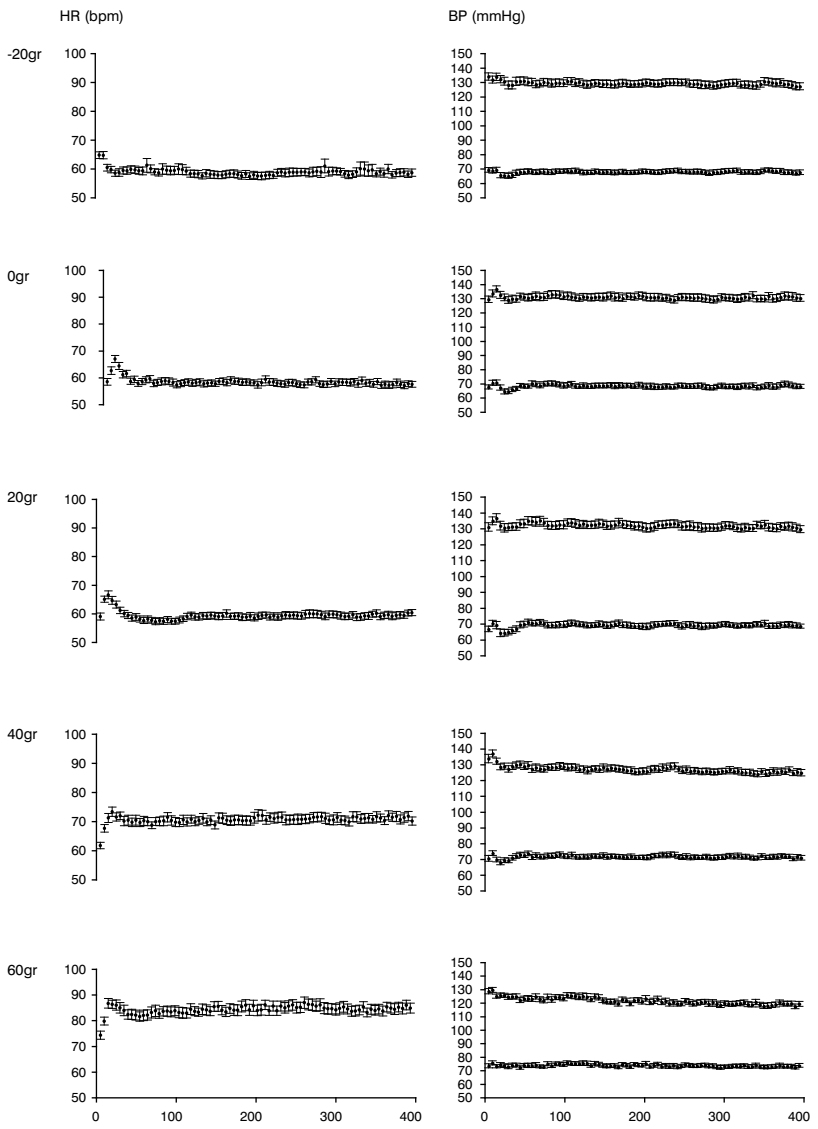
83%, respectively. In the first minute, 81% of the diastolic BP measurements stabilized in supine position and 92% at 60 degrees HUT. After 4 minutes, all variables measured could be considered stable, irrespective of body posture.

Figure 1



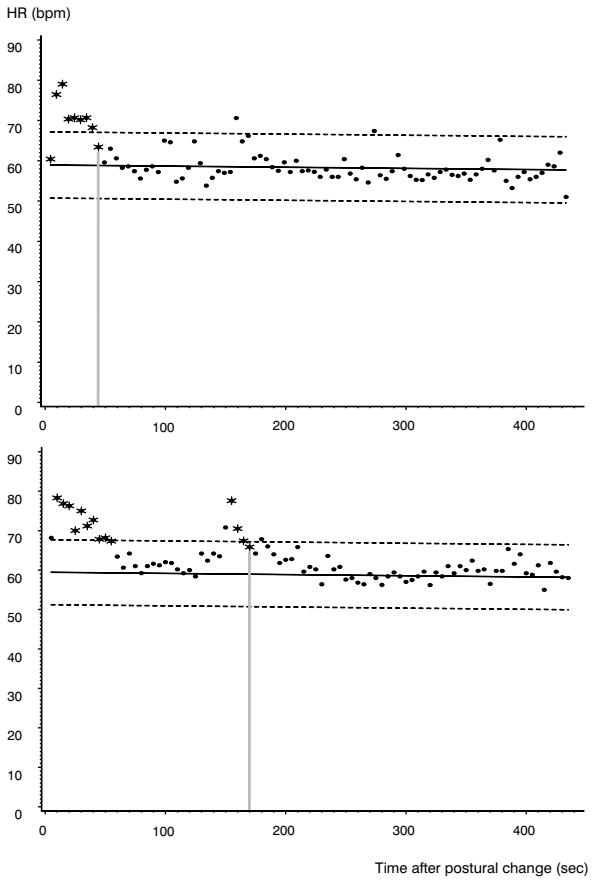
The individual profiles of heart rate (HR) and mean arterial pressure (MAP) during the 7 minutes in supine position at each consecutive assessment.

Figure 2



Heart rate (HR) and blood pressure (BP) at 5-seconds intervals at each rotational step. Data represent the overall mean at each time point and vertical bars represent the SEM.

Figure 3



Heart rate profiles of 2 participants at 20 degrees head-down tilt with different time needed to reach stability after rotation. Solid line represents individual regression line and broken lines represent 90% confidence band, using a linear mixed model. Stars relate to period of instability. The last star in time indicates the start of the period of stability (grey line).

Third, although we observed relatively high variations within subjects in autonomic function, there were no differences between PERIOD5, PERIOD6 and PERIOD7, in which the spectral analyses were performed, regardless of the rotational step (Figure 5).

Table 2 Standard deviation and coefficient of variation (%) of the autonomic variables during head-up tilt (HUT), using linear mixed model.

		-20		0		20		40		60	° HUT
LF pi	BS	266	(31.9%)	250	(20.9%)	299	(26.8%)	574	(45.8%)	511	(47.4%)
	BA	14	(1.7%)	99	(8.3%)	146	(13.1%)	250	(20.9%)	75	(7.0%)
	WS	480	(57.5%)	574	(47.9%)	548	(49.1%)	517	(43.3%)	389	(36.1%)
HF pi	BS	1882	(55.7%)	1786	(59.6%)	1471	(63.9%)	734	(86.7%)	243	(71.1%)
	BA	0	(0%)	96	(3.2%)	220	(9.6%)	35	(4.1%)	32	(9.4%)
	WS	1707	(50.6%)	1060	(35.4%)	577	(25.1%)	230	(27.2%)	115	(33.7%)
LF/HF	BS	0.3	(70.8%)	0.5	(71.4%)	0.5	(72.1%)	1.3	(60.8%)	3.1	(70.9%)
	BA	0	(0%)	0	(0%)	0.1	(9.3%)	0.5	(20.6%)	0.8	(19.5%)
	WS	0.2	(39.1%)	0.4	(52.1%)	0.4	(45.4%)	1.0	(43.9%)	1.6	(36.0%)
LF sys	BS	2.5	(36.9%)	4.6	(46.9%)	6.7	(56.1%)	12.8	(78.1%)	18.7	(81.9%)
	BA	0.4	(24.3%)	0	(0%)	2.5	(20.5%)	5.4	(33.1%)	2.96	(12.9%)
	WS	6.6	(95.9%)	4.8	(48.2%)	5.4	(44.6%)	10.8	(65.7%)	7.66	(33.5%)
BRS	BS	2.6	(21.4%)	3.5	(28.5%)	2.6	(23.7%)	1.3	(15.5%)	1.5	(22.6%)
	BA	0	(0%)	0.3	(2.6%)	0.9	(7.8%)	0	(0%)	0.3	(5.1%)
	WS	4.3	(35.3%)	3.2	(25.5%)	2.2	(19.9%)	1.8	(21.4%)	2.0	(29.8%)

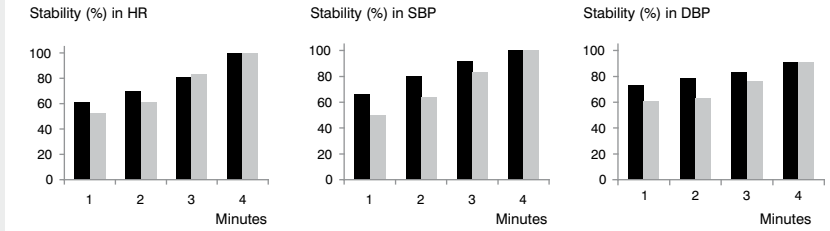
LF pi respectively HF pi: power of the low respectively high frequency component of the fluctuations in pulse interval, LF/HF: ratio between LF pi and HF pi, LF sys: low frequency component of the fluctuations in systolic blood pressure, BRS: baroreflex sensitivity, BS: variation between subjects, BA: variation between assessments, WS: variation within subjects.

Discussion

We studied the various aspects and influencing factors of the reproducibility of the pattern of the hemodynamic and autonomic response during graded head-up tilt in young, healthy women. Heart rate, mean arterial blood pressure and sympathetic activity increased and baroreflex sensitivity decreased. This response to orthostatic stress in all women was consistent with other reports[118;167;168].

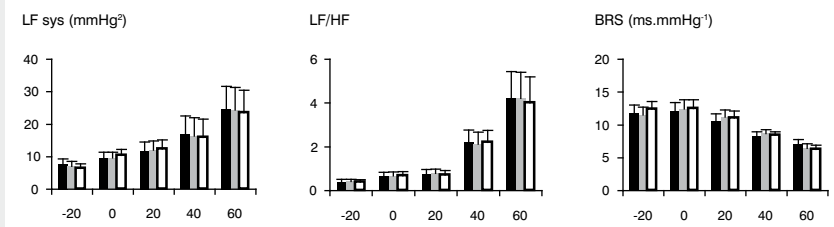
The reproducibility of the hemodynamic and autonomic variables during head-up tilt was relative good as we observed a relative low variation in blood pressure and

Figure 4



The (cumulative) percentage of all profiles of all participants having reached stability in heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) at each minute after rotational change. Black and grey bars represent supine position and +60 degrees head-up tilt, respectively.

Figure 5



Autonomic variables, calculated for the 3 periods during head-up tilt (HUT). Black, grey and white bars represent the mean values for the period 0-5 minutes, 1-6 minutes and 2-7 minutes, respectively. Vertical lines represent SEM. LFsys: (vascular) sympathetic activity, LF/HF: ratio of low and high frequency component of the fluctuations in pulse interval (cardiac autonomic balance), BRS: baroreflex sensitivity.

heart rate during the graded head-up tilt maneuver (CV between assessments below 4%). Between assessments variation in autonomic function was acceptable (CV below 35%). Due to differences in methodology, comparison amongst studies

is difficult[137;143-145;164;169-173]. Most studies have evaluated the variation in mean heart rate and blood pressure during two or more occasions[143;145]. Studies on autonomic function by using spectral analysis are generally performed at rest and reported moderate reproducibility, with baroreflex sensitivity showing the best results[143;171]. In our study, the differences in variation between assessments between subjects and within subjects were separately analyzed at each rotational step, using the complete set of data points. We feel that this is a more reliable method to determine variation in beat-to-beat data and allows an optimal assessment of reproducibility. Increasing tilt angles during HUT did not induce an increasing variation in blood pressure and heart rate. In addition, we observed no differences between the morning- and afternoon-assessments. These results indicate that blood pressure and heart rate recordings are relatively stable during various situations, creating an appropriate basis for using spectral analysis to assess autonomic function. Because of the low variation between assessments, but relatively high variation between and within subjects in the various autonomic variables, this method is especially useful in studies using repeated measures design.

Orthostatic stress testing can be applied in many disease states, such as diabetes, cardiovascular disease, postural tachycardia syndrome and hypertension. As in most studies the differences between groups or after repeated measures are larger than the variation we observed in this study[167;174-178], enclosure of an orthostatic maneuver into the assessment seem to enhance the reproducibility of autonomic function testing.

In the third part of the study, we studied the contribution of including the acute hemodynamic changes that occurred after each rotational into the steady state data segments. There is currently no conclusive method that describes the exact duration that is required to reach steady state conditions. These time periods vary in the literature and are different for active and passive postural change[162;163;179-182]. This is the first study in which the point of stability was exactly calculated for every single participant, in each condition. We observed that the major part reaches stability within one minute. However, it needs to be stressed that, still, there was a slight increase till 4 minutes, either due to a delay in hemodynamic stability or a possible second period of instability (participant 2 in Figure 3). We feel that using this method gives an accurate indication of the duration and its variation among

subjects of the hemodynamic changes directly after rotational change. This more precise calculation may be useful in studies on the hemodynamic and autonomic changes during head-up tilt.

Finally, we hypothesized that the time needed to adjust to postural change would influence spectral analysis results, but autonomic function estimates turned out to be similar when started directly as compared to 1 or 2 minutes after rotation. Therefore, we can conclude that neither the drift in heart rate nor the immediate changes after postural change affected our spectral analysis results. These observations imply that the spontaneous fluctuations in heart rate and blood pressure, directly recorded after postural change can be used for spectral analysis.

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We determined autonomic function by use of spectral analysis, which is a non-invasive, well validated technique and frequently used during HUT[120;137-139]. Although this method has limitations when used as single point measurement[140;141], our data indicate that this technique is especially suitable for studies requiring repeated measures. Moreover, there are indications that changes in blood pressure variability correspond well with concomitant changes in muscle sympathetic nerve activity[142].

We studied a group of healthy, young, female participants. Both gender and age are known to influence cardiovascular variability assessed by spectral analysis[183-185]. The low variation in blood pressure may be at the expense of large fluctuations in sympathetic activity in young people, to keep blood pressure stable. We studied a group of healthy, young women to determine their autonomic response to orthostatic stress as pregnancy confines to this group. Moreover, these data can also serve as reference values for responses observed in formerly preeclamptic women, which are reported to have alterations in autonomic blood pressure control[6].

We did not perform assessments at paced breathing. However, considering the low variation between assessments we think that respiration has had only minor influence on our results. Other also reported that beat-to-beat HR and BP variations are not greatly influenced by respiratory activity, but mostly depends on other factors, likely of neural nature[186;187].

In summary, graded head-up tilt is an effective test to reproducibly assess the response pattern to orthostatic stress, to determine the sensitivity of the hemodynamic and autonomic system. Spectral analysis can be performed on heart rate and blood pressure data recorded directly after postural change as it is not affected by the period needed to reach (hemodynamic) stability after rotation.

Chapter 5

The relation between venous reserve capacity and low plasma volume

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Abstract

Pre-pregnant low plasma volume (LPV) is associated with subsequent gestational hypertensive disease. It is unknown to what extent a LPV affects the venous reserve capacity (VRC). We tested the hypothesis that LPV reduces the VRC, as indicated by pre-syncope (FAINT) or altered cardiovascular changes in response to head-up tilt.

In 52 non-pregnant women with a history of preeclampsia or recurrent miscarriage we assessed plasma volume, stroke volume and cardiac output and determined blood pressure, heart rate and autonomic responses to stepwise inflicted head-up tilt. Twelve participants (23%) had LPV, which related to FAINT (adjusted OR 5.9, 95% CI 1.2-29.6) as compared to subjects with normal plasma volume (NPV). Women with LPV without FAINT demonstrated a circulatory response comparable to NPV-women at the expense of consistently higher heart rate. LPV decreases the capacity to cope with head-up tilt without affecting the response pattern, suggesting reduced venous reserve capacity.

Introduction

Gestational hypertensive complications has been related to pre-existing hypertension, renal disease, diabetes mellitus, thrombophilia, recurrent miscarriage and preeclampsia in a previous pregnancy[2;3]. Moreover, formerly preeclamptic women are at increased risk to develop cardiovascular diseases in later life[112].

Low plasma volume is a common characteristic of preeclampsia. In part of these women plasma volume remains subnormal after delivery, without compensatory regulatory changes in active plasma renin, angiotensin II, atrial natriuretic peptide and (nor)epinephrine levels[4;188]. This condition is associated with reduced venous compliance, and higher resting sympathetic activity along with reduced baroreflex sensitivity[5;6]. Clinically, low plasma volume prior to pregnancy predisposes to early-pregnancy hemodynamic maladaptation, gestational hypertension, preeclampsia and fetal growth restriction in a subsequent pregnancy[8].

Functionally, low plasma volume may affect the venous reserve capacity. Under basal conditions 2/3 of the plasma volume is localized in the venous compartment. About 60% is hemodynamically inactive (unstressed volume) and reflects the venous reserve capacity. The unstressed volume is mobilized in situations when the demand is increased, such as in physical exercise or during orthostasis. During exercise, women with persistently low plasma volume demonstrate a reduced ability to raise stroke volume, leaving cardiac output primarily modulated by changes in heart rate[114]. Likewise, this condition may also influence orthostatic tolerance.

Positive head-up tilt induces an initial decrease in venous return, which negatively affects cardiac output. To ascertain adequate venous return, a compensatory increase in hemodynamically active (stressed) volume is needed at the expense of the unstressed volume, through venoconstriction. In this reasoning, low plasma volume may negatively affect the cardiovascular response to postural changes. To date, most studies on plasma volume and orthostatic tolerance have been performed under acutely induced changes in circulatory volume, a situation that always affects neuro-humoral control mechanisms[168;189]. In contrast, plasma volume is chronically reduced in many formerly preeclamptic women and volume regulatory hormones are comparable to healthy parous controls[4;188]. We hypothesize that normotensive formerly

preeclamptic women and women with recurrent miscarriage with low plasma volume exhibit less tolerance to head-up tilt, as indicated by imminent fainting or altered cardiovascular changes in response to head-up tilt. To test this hypothesis, we subjected women with LPV and NPV to passive stepwise head-up tilt and measured blood pressure, heart rate, stroke volume, cardiac output and autonomic function.

Methods

Subjects

Between April 2005 and March 2006, 136 women were referred to the outpatient clinic of the Department of Obstetrics of the Radboud University Nijmegen Medical Centre for post-gestational follow up after preeclampsia or recurrent miscarriage. Eighty-four women were excluded from analysis because of hypertension (resting systolic blood pressure (SBP) > 140 mmHg and/or diastolic blood pressure (DBP) > 90 mmHg) and/or the use of anti-hypertensive medication. Fifty-two consecutive normotensive, non-pregnant women with a history of pre-eclampsia (PE) with or without HELLP-syndrome (hemolysis, elevated liver enzymes, low platelets) or recurrent miscarriage (RM), between 24 and 42 years, were included in the study. All women were more than 6 months after their last pregnancy, not breastfeeding and not taking any medication.

PE and HELLP-syndrome were defined according to the criteria of the International Society on the Study of Hypertension in Pregnancy (ISSHP)[190]. RM was defined as two or more spontaneous fetal losses before 16 weeks' gestation. Informed consent was obtained from all participants and the study was approved by the Institutional Review Board.

Experimental design

Prior to the head-up tilt test (HUT), all women underwent plasma volume measurement and echocardiographic evaluation, which started at 10 AM, as outlined in Figure 1. Participants and researchers were blinded to these findings at the time of HUT.

Head-up tilt was performed after voiding the bladder and executed under controlled environmental conditions, in a quiet and partially darkened room with an ambient temperature of 26 °C[116]. Subjects were positioned on the tilt table on a comfortable

mattress to minimize muscular activity, and both arms were positioned so that they remained at heart level during tilting. Participants remained supine for 10 minutes, thereafter head-up tilt was imposed by passively changing the body posture from 20 degrees head-down (-20°) to 60 degrees head-up tilt (+60°), in steps of 20° at 10 minutes intervals.

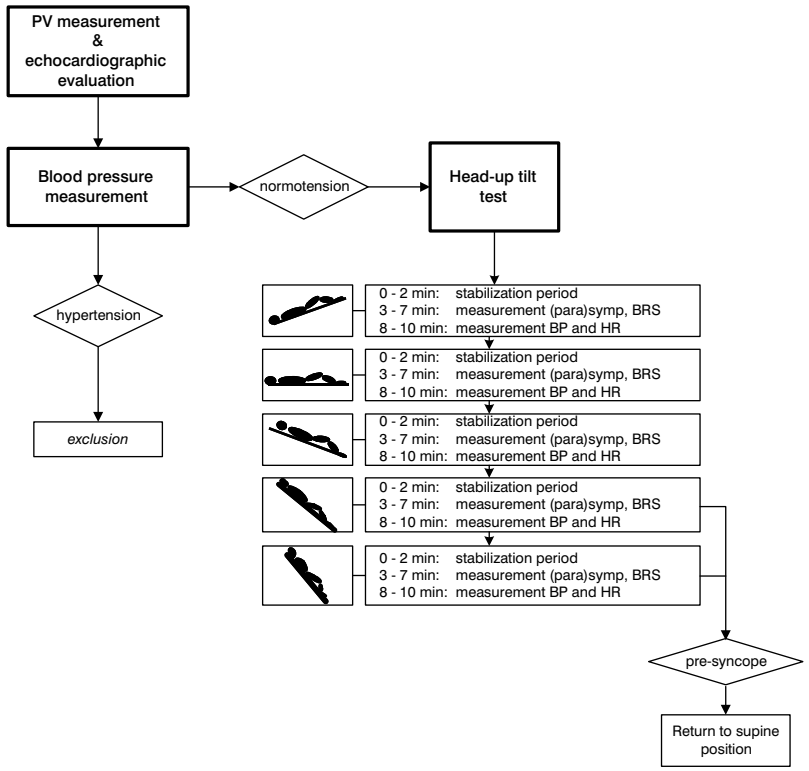
Measurements

Plasma volume (PV) was measured using the Iodine^{125} albumin indicator dilution method (^{125}I-HSA) and was expressed in milliliters per kilogram lean body mass (LBM). Lean body mass was calculated, not measured[4]. LPV was defined as a PV < 49 ml·kg$_{LBM}^{-1}$, which is two standard deviations below the mean as reported for healthy parous controls[4].

Baseline echocardiographic measurements were obtained by an experienced cardiology technician, who measured the left atrial diameter, the left ventricular outflow tract velocity integral, and the left ventricular outflow tract diameter. Measurements were performed in left lateral position, using a cross-sectional phased array echocardiographic Doppler system (Vivid 7, General Electric, Horten, Norway). Heart rate (HR) was determined by the reciprocal of the RR-interval of the ECG measured during the echo Doppler measurements. Stroke volume (SV) was calculated by multiplying the left ventricular outflow tract velocity integral and the left ventricular outflow tract diameter. Cardiac output (CO) was calculated as SV*HR and total peripheral vascular resistance (TPVR) as eighty times the mean arterial pressure (MAP), divided by the cardiac output (80*MAP/ CO). The index values were calculated by dividing the SV, CO and TPVR by the body surface area (BSA), as determined by the method described by Du Bois and Du Bois[191].

During HUT, hemodynamic values were obtained in steady state at each rotational step (Figure 1). Steady state was defined after the first 2 minutes of each rotational step[164;165]. Blood pressure (BP) and HR were measured at the left upper arm (Dinamap, Critikon, Florida, USA). Fluctuations in HR and arterial BP (ABP) were measured continuously, by a finger ABP-monitoring device attached to the 3rd digit of the right hand at a sampling rate of 100 Hz (Finometer, Finapres BV, The Netherlands), to determine sympathetic activity, parasympathetic activity and baroreflex sensitivity. We derived relative brachial pressure from the finger arterial

Figure 1



Flowchart of experimental protocol. PV: plasma volume, (para)symp act: (para)sympathetic activity, BRS: baroreflex sensitivity, BP: blood pressure, HR: heart rate.

pressure by the application of waveform filtering and level correction. Relative changes in SV and CO were determined by continuous beat-to-beat pulse contour analysis from the Finometer, as previously validated[192]. The changes in SV and CO were transformed to absolute values, by relating them to the values measured by echocardiography at rest prior to the test.

We quantified autonomic activity and baroreflex sensitivity by spectral analysis technique[120]. The recordings were subdivided into data segments of 100 s, overlapping for 50%, and resampled at 5.12 Hz. Each segment was then analyzed

with a Fast Fourier Transformation that searches for rhythmic fluctuations in SBP and pulse interval (PI) with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. Subsequently, the SBP and PI powers were expressed as a function of the frequency. Sympathetic activity was defined as the natural logarithm of power of the low frequency (LF) component of the variations in SBP and the ratio of absolute LF and high frequency (HF) powers of the PI was assumed to represent the autonomic balance between the sympathetic and vagal system. Baroreflex sensitivity (BRS), which provides information about the changes in HR (output) in response to fluctuations in SBP (input), was defined as the (low frequency) transfer gain from SBP to PI and expressed in $ms.mmHg^{-1}$.

Some women experienced pre-syncope and could not complete the whole HUT. Pre-syncope was defined by symptoms of light-headedness, blurred vision, dizziness and/or nausea. In women who experienced pre-syncope, it was not possible to obtain steady state recordings of BP and HR at the final head-up tilt step. Therefore, the data of the women without and with pre-syncope were analyzed separately. As the oscillometrical blood pressure measurements were performed at the end of each rotational step and were unavailable in the women with pre-syncope, for the sub-analysis of those women, we used the Finometer measurements for BP and HR, and not the oscillometrically determined blood pressure measurements, to describe the hemodynamic changes relative to those in supine position.

Statistical analysis

Data are presented as means ± standard errors (SE) of the mean for continuous data, as well as median and ranges for interval variables. Differences between groups, with respect to basal values and responses to head-up tilt, were analyzed non-parametrically using Mann Whitney *U* test or Fischer Exact Test whenever applicable. For each group, we quantified the responses to head-up tilt in HR, SV, MAP, sympathetic activity, autonomic balance and baroreflex sensitivity, by calculating the area-under-the-curve and by linear regression analysis.

To identify the independent risk factors for the susceptibility to low plasma volume and to imminent fainting, we performed a multivariate backward stepwise logistic regression analysis. We calculated both the Mantel-Haenszel odds ratio as the adjusted odds ratio for imminent fainting. A p-value of less than 0.05 was considered significant.

Results

Forty (77%) of the 52 normotensive women had NPV (median 56, range 49-77 ml·kg$_{LBM}^{-1}$) and 12 (23%) LPV (median 47, range 43-48 ml· kg$_{LBM}^{-1}$). The demographic characteristics of these two groups of women are listed in Table 1. Age, body mass index, smoking and obstetric history were not different between the two groups. In resting supine position, there were no differences in MAP and TPVR. Women with LPV had a lower resting stroke volume (-12%) and smaller left atrial diameter (-10%) than the women with NPV.

Table 1 Demographic characteristics of women with normal plasma volume (NPV) and low plasma volume (LPV).

	NPV	LPV	p value
Number of women	40	12	
Age (yr)	34 (27-42)	31 (25-38)	ns
BMI (kg·m^{-2})	24 (18-38)	25 (20-33)	ns
Obstetric history:			
- recurrent miscarriage	11 (26%)	6 (50%)	ns
- PE and/or HELLP syndrome	29 (74%)	6 (50%)	ns
Smoking	5 (13%)	2 (20%)	ns
Mean arterial blood pressure (mmHg)	82 (66-103)	85 (69-96)	ns
Left atrial diameter (mm)	40 (35-48)	35 (31-47)	<0.01
Stroke volume (ml)	67 (45-119)	59 (43-82)	<0.05
Stroke volume index (ml·m^{-2})	38 (28-55)	32 (27-42)	<0.05
Heart rate (bpm)	68 (52-82)	74 (60-93)	<0.05
Cardiac output (l·min^{-1})	4.3 (3.1-8.3)	4.3 (3.0-5.9)	ns
Cardiac index (l·min^{-1}·m^{-2})	2.4 (1.9-4.0)	2.3 (2.0-3.4)	ns
TPVR (dyne·s·cm^{-5})	1455 (790-2194)	1511 (1275-2320)	ns
TPVR index (dyne·s·cm^{-5}·m^{-2})	838 (364-1337)	812 (621-1532)	ns

Values presented as median (ranges) or number (percentage).
ns: not significant, BMI: body mass index, PE: pre-eclampsia, HELLP: haemolysis, elevated liver enzymes, low platelets syndrome, TPVR: total peripheral vascular resistance.

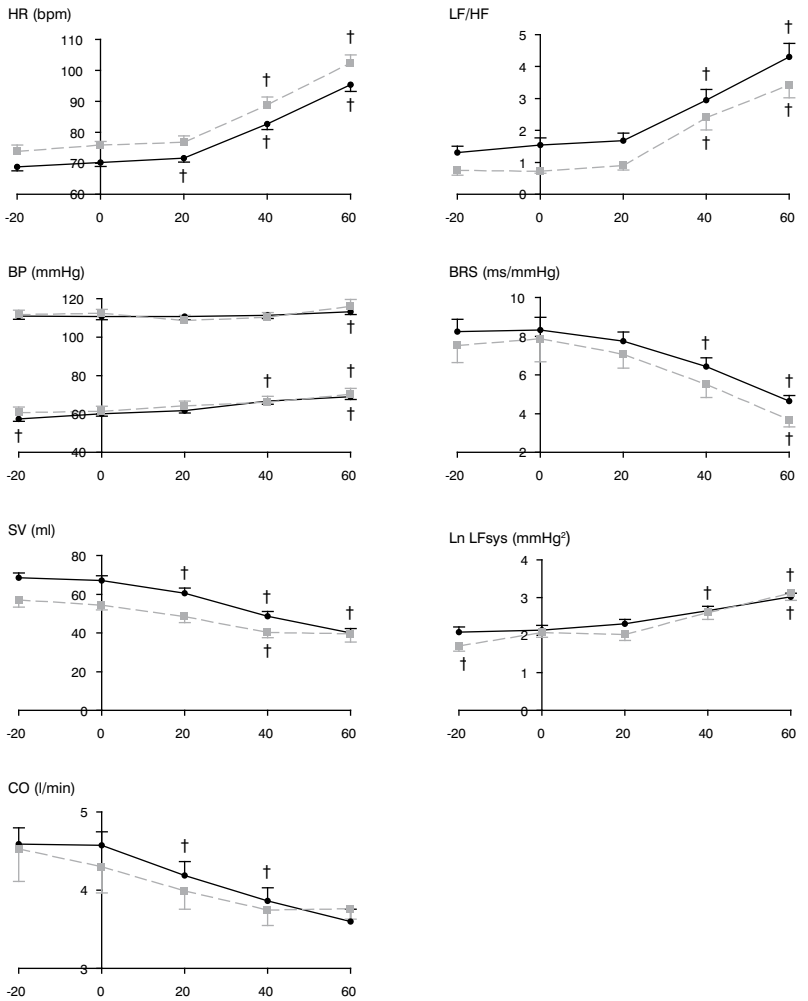
Multivariate regression analysis showed that the susceptibility to low plasma volume was not affected by BMI, smoking and obstetric history.

Nine women experienced pre-syncope; 4 had NPV (4/40; 10%) and 5 had LPV (5/12; 42%), indicating an increased chance of pre-syncope in women with LPV (Mantel-Haenszel odds ratio: 6.4, 95% CI 1.4-30.1). After performing multivariate regression analysis with BMI, obstetric history and plasma volume as covariates, only plasma volume remained as an independent variable for imminent fainting (adjusted odds ratio 5.9, 95% CI 1.2-29.6).

The responses to HUT in the women without pre-syncope are shown in Figure 2. In the 36 women with NPV, we observed no changes with head-down tilt. With head-up tilt, a step-wise progressive increase was observed between 0° and 60° in HR (+25 ± 2 bpm; p<0.01), SBP (+2.5 ± 1 mmHg; p=0.02), DBP (+9 ± 1 mmHg; p<0.01) and TPVR (+623 ± 52 dyne·s·cm^{-5}; p<0.01) and a decrease in SV and CO, with maximal changes at 60° head-up tilt of –27 ± 2 ml (p<0.01) and –0.9 ± 0.1 l·min^{-1} (p<0.01). Sympathetic activity and autonomic balance increased during head-up tilt (24.3 ± 2.4 mmHg^2, 2.8 ± 0.4 units (p<0.01), respectively) while baroreflex sensitivity decreased (-3.7 ± 0.7 ms·mmHg^{-1}, p<0.01). The seven women with LPV demonstrated a similar pattern of changes in response to HUT as the NPV women, except that HR was consistently higher and SV lower during head-up tilt than observed in NPV-women (area-under-the-curve 1671 ± 32 versus 1555 ± 27, p=0.03 respectively 958 ± 48 versus 1139 ± 47, p=0.04). During head-up tilt the sympathetic activity, autonomic balance and baroreflex sensitivity of the LPV-women were not different from those in NPV-women (Figure 2).

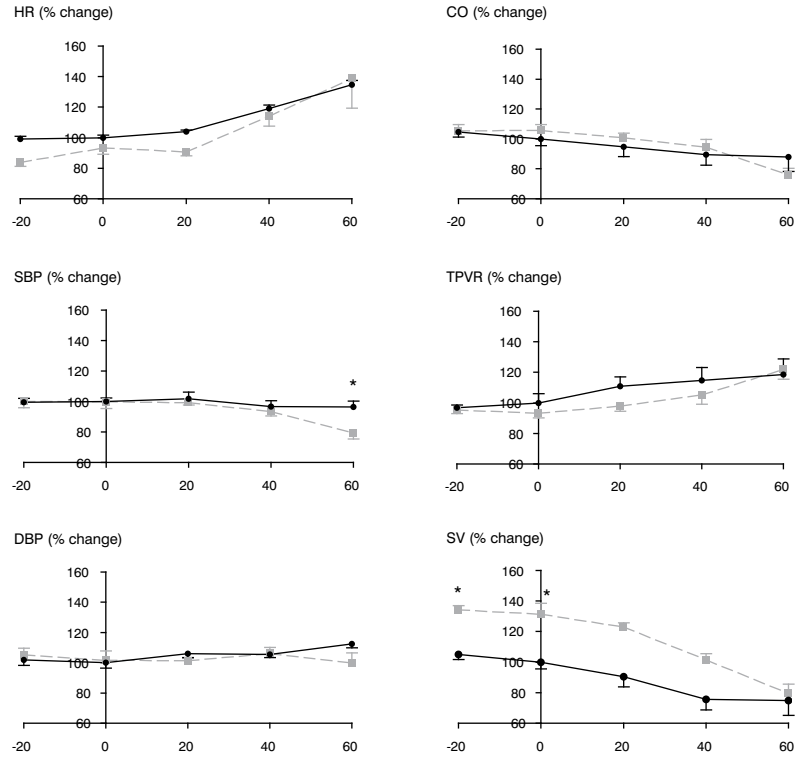
From the 12 women with LPV, five women experienced pre-syncope during HUT. Pre-syncope occurred at 40° head up tilt in one woman and at 60° in the other four women. After return to the supine position, HR and BP normalized within one minute in all women. Figure 3 shows the relative changes in response to head-up tilt from the supine position for the women with LPV, with and without pre-syncope. The five women with pre-syncope demonstrated a similar pattern of changes in response to HUT as the women without pre-syncope, except for a steeper decline in SV with progressive tilt (regression coefficient –0.60 ± 0.08 versus -0.28 ± 0.08, p=0.03). The steep decline was noticeable already at the lower levels of tilt. The 4 women

Figure 2



Hemodynamic and autonomic changes (mean ± SEM) in response to head-up tilt in 43 women without pre-syncope: 7 women with low plasma volume (--■--) and 36 women with normal plasma volume (–●–). HR: mean heart rate, BP: blood pressure (systolic and diastolic), SV: stroke volume, CO: cardiac output, LF/HF: autonomic balance, BRS: baroreflex sensitivity, Ln LF sys: sympathetic activity (ln LF of the spontaneous variation in systolic blood pressure). † : $p<0.05$ as compared to supine.

Figure 3



Hemodynamic changes ± SEM in response to head-up tilt in 12 women with low plasma volume: 5 women with pre-syncope (--■--) and 7 women without pre-syncope (–●–). HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, CO: cardiac output, TPVR: total peripheral vascular resistance, SV: stroke volume. * $p<0.05$ between groups.

with NPV and pre-syncope had similar changes in BP and HR prior to the collapse as the comparable subgroup with LPV. The responses of all measured variables on head-up tilt were similar in women with a history of preeclampsia compared to women with a history of recurrent miscarriage.

Discussion

In this study, we hypothesized that low plasma volume represents reduced venous reserve capacity, which negatively affects the response to head-up tilt. Our findings seem to support the hypothesis since women with LPV subjected to head-up tilt experience a higher rate of pre-syncope than women with NPV.

The response to head-up tilt in women with NPV without FAINT was consistent with other reports[118;167;168]. Women with LPV who could tolerate HUT showed a consistently higher HR and a lower SV, but no differences in response pattern, during head-up tilt as compared to the NPV-group. These results suggest a resetting of the hemodynamic system in LPV-women towards a higher sympathetic level without affecting the magnitude of rapid corrective autonomic responses.

Women who experienced pre-syncope were apparently unable to ensure sufficient cerebral perfusion as indicated by symptoms of pre-syncope at higher levels of head-up tilt. Several compensatory mechanisms ensure cerebral perfusion. First, cardiac output rises at the expense of unstressed blood volume mobilized by venous constriction and by an increase of cardiac contractility. If this first line of defense is insufficient, arterial redistribution reroutes the blood flow towards the brain. In women with LPV both resting venous compliance and capacitance are reduced[4;7]. This suggests an already activated compensatory venous contractile state or reduced venous dimensions, without capability of further venoconstriction. These findings are in line with the inability to raise SV as reported in response to cycling in LPV-women[114].

A steeper decline in SV during increasing head up tilt was the only preceding sign in women with pre-syncope. The data during the final step of tilt in which pre-syncope occurred suggest insufficient cardiac preload that initiates a rapid unbalanced further decline in venous return. This is probably the consequence of parasympathetic dominance and collapse through the Bezold-Jarisch reflex, which is initiated through stimulation of the ventricular afferents when filling pressures are too low[193].

The phenotype 'low plasma volume' may originate from shallow venous development as part of a 'fetal origin of disease' complex within the Barker hypothesis[194]. On the other hand, it may also be the resultant of sympathetic overactivity, such as in the metabolic syndrome, giving the treatment opportunity to sympathetic blocking medicaments or life style adjustments.

In this study, we included both women with a history of PE as women with a history of RM. Women with RM may be at increased risk for preeclampsia[3] and we speculated that circulatory dysfunction is common amongst those women. We observed a similar incidence of LPV and FAINT in formerly preeclamptic women as in women with RM, but this might be due to small sample size. However, multivariate regression analysis showed that the susceptibility to LPV or to FAINT was not influenced by obstetric history.

Additionally, we planned to investigate the involvement of plasma volume in the cardiovascular response to head-up tilt, to study the importance of the venous compartment in our study-group. The response to head-up tilt of an additional group of normal parous controls would only have illustrated if our results were due to the plasma volume status independent of the complicated obstetric history. As we defined low plasma volume on base of the plasma volume levels in women with an uncomplicated obstetric history, we expected that the response of those women mimic the results of the NPV-group. This view is supported by our previous findings on the similarities in circulatory responses to alterations in vascular resistance between healthy parous controls and formerly preeclamptic women with normal plasma volume[4;11;188].

The level of physical fitness was not measured prior to the study. The latter is known to be correlated with both plasma volume and sympathetic tone[195]. Although we have no reason to assume a difference in physical condition between groups, a reduced physical condition could have influenced the number of women with a low plasma volume or increased sympathetic activity.

We tested all women after a comparable evolved time period of at least 6 months post-gestationally and all participants did not express any physical complaints, but it may be possible that our findings are affected by the current health status and/or the former (preeclamptic) pregnancy. As we could not substantiate historical differences between groups, we do not think that this factor influenced the results.

We analyzed women with pre-syncope separately from those who could tolerate HUT. Pre-syncope was defined by subjectively reported symptoms. Theoretically, some women having the same complaints may have refrained from telling. As women with pre-syncope could also be identified by an increasing heart rate and/or decreasing blood pressure during their final head-up tilt step when complaints occurred, we do not think that this subjectivity has affected the results[196].

In conclusion, our data indicate that LPV is associated with an increased chance of pre-syncope during head-up tilt. Women with LPV who do not faint upon head-up tilt are able to compensate for the reduction in SV by a consistently higher HR. These data suggest a resetting to a chronically-elevated sympathetic tone in women with low plasma volume along with a reduction in venous reserve capacity.

Chapter 6

Venous response to orthostatic stress in formerly preeclamptic women

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Submitted

Abstract

Objective: We hypothesized that the venous reserve capacity, evaluated by autonomic and/or venous responsiveness to head-up tilt (HUT), is compromised in formerly preeclamptic women with low plasma volume (PV).

Study Design: In 30 women, we assessed PV and determined venous compliance (VeC) and sympathetic activity (SYMP) during graded HUT. Participants were divided into three groups based on their PV: low (LPV), medium (MPV) and high (HPV). Differences were analyzed non-parametrically.

Results: HUT reduced VeC and increased SYMP. PV correlated with supine VeC ($r=0.64$, $p<0.01$) and with the VeC-response to HUT ($r=0.59$, $p<0.01$). During HUT, LPV-women showed a smaller decline in VeC and a lesser rise in SYMP as compared to the MPV- and HPV-groups.

Conclusion: Low plasma volume is related to reduced venous reserve capacity. This may be caused by a small or pre-contracted venous compartment, or indicate sympathetic incapability to further constrict the venous system during HUT.

Introduction

Under normal circumstances, about two third of the plasma volume is localized in the venous compartment. About 60% is hemodynamically inactive and reflects unstressed volume[26]. The venous reserve capacity is the ability of the venous system to mobilize unstressed volume.

At positive head-up tilt, an initial decrease in venous return negatively affects cardiac output and blood pressure. A compensatory sympathetically controlled decrease in venous compliance and unstressed volume re-establishes venous return[12]. Therefore, adequate functioning of the venous system can be exemplified by the response to orthostatic stress.

In about half of women with a history of preeclampsia, plasma volume remains subnormal after pregnancy[4;8]. Low plasma volume seems to reflect reduced venous reserve capacity, as indicated by the diminished ability to raise stroke volume during physical activity[114] and blunted hemodynamic and autonomic responses to a reduction in preload during orthostatic stress[197]. In these women, during cycling, changes in cardiac output are primarily modulated by changes in heart rate. Likewise, during head-up tilt, women with low plasma volume respond with lower stroke volume and consistently higher heart rates as compared to their normal plasma volume counterparts. Moreover, they are vulnerable to pre-syncope during tilt. Whether the apparent incapacity to mobilize unstressed volume originates from shallow venous wall response capacity or altered autonomic regulation is currently unknown.

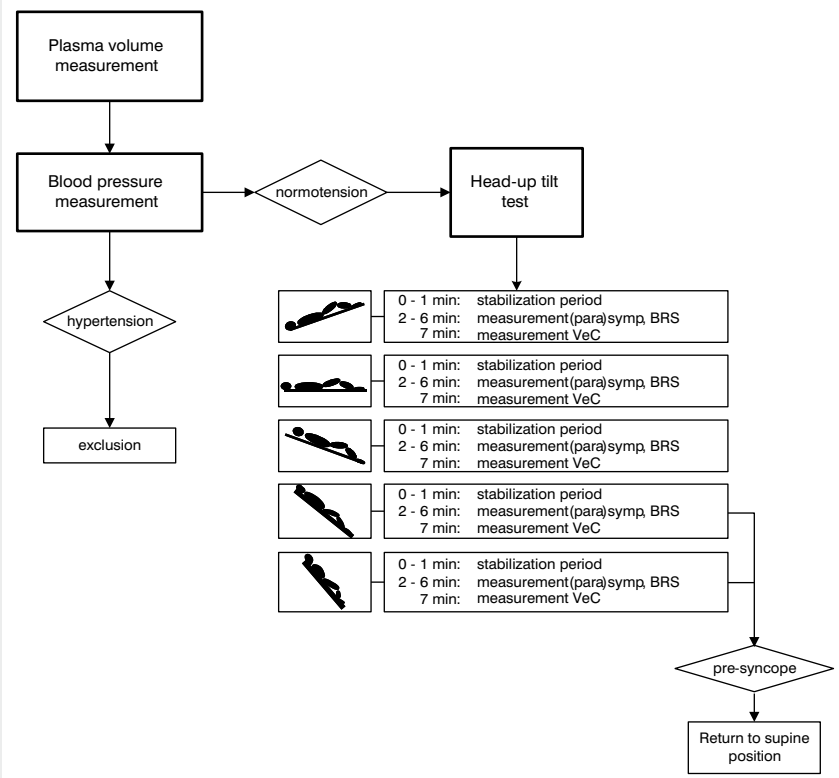
In this study, we tested the hypothesis that the reduced venous reserve capacity in women with low plasma volume is caused by blunted venous and/or autonomic responsiveness. To this end, we compared the changes in venous compliance and vascular sympathetic activity during positive head-up tilt in formerly preeclamptic women with low, medium and high plasma volume.

Methods

Subjects:

Thirty non-pregnant women with a history of pre-eclampsia (PE) with or without HELLP-syndrome (hemolysis, elevated liver enzymes, low platelets), between 22 and 38 years, were included in the study. All women were recruited from the outpatient clinic of the Department of Obstetrics of the Radboud University Nijmegen Medical Centre for post-gestational follow up after preeclampsia. Women with hypertension (resting systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg) and/or the use of anti-hypertensive medication were excluded. All women were at least 6 months post-partum, not breastfeeding and not taking any medication. PE and HELLP-syndrome were defined according to the criteria of the International Society on the Study of Hypertension in Pregnancy (ISSHP)[190]. The study was approved by the Institutional Review Board (CMO nr. 2006/245) and written informed consent was obtained from all participants.

Figure 1



Flowchart of the experimental protocol. (para)symp act: (para)sympathetic activity, BRS: baroreflex sensitivity, VeC: venous compliance.

Experimental design:

Prior to the head-up tilt test (HUT), all women underwent plasma volume measurement that started at 10 AM, as outlined in Figure 1. Participants and researchers were blinded to these results at the time of HUT.

Head-up tilt was performed after voiding the bladder and executed under controlled environmental conditions, in a quiet and partially darkened room with an ambient temperature of 26 °C[116]. Subjects were positioned on the tilt table on a comfortable mattress to minimize muscular activity, and both arms were positioned at heart level. Participants remained supine for 10 minutes, thereafter head-up tilt was imposed by passively changing the body posture from 20 degrees head-down (-20°) to 60 degrees head-up tilt (+60°), in steps of 20° at 10 minutes intervals. Subjects were asked to refrain from smoking, caffeine and alcohol at least 10 hours prior to the tilt test.

Measurements:

Plasma volume (PV) was measured using the Iodine^{125} albumin indicator dilution method (^{125}I-HSA) and was expressed in milliliters per kilogram lean body mass (lbm). Lean body mass was calculated using the method of Deurenberg, *et al* [4]. For purposes of comparison, all women were subdivided into three groups, based on their plasma volume; low (LPV), medium (MPV) and high (HPV) plasma volume group.

Systolic, diastolic and mean arterial blood pressure (SBP, DBP and MAP) and heart rate (HR) were measured at the left upper arm (Dinamap, Critikon, Florida, USA). During HUT, hemodynamic and autonomic values were obtained in steady state at each rotational step (Figure 1). During HUT, hemodynamic and autonomic values were obtained in steady state at each rotational step (Figure 1).

Autonomic function was derived from spontaneous fluctuations in blood pressure and heart at steady state. Head-up tilt induces immediate hemodynamic changes[26]. Previous experiments in our laboratory have shown that a dynamic time course of less than 60 seconds is needed to reach a new steady state after postural change in the majority of measurements. In the present setup we excluded the data of the first minute after changing position. Fluctuations in HR and arterial BP (ABP) were measured continuously, using a finger ABP-monitoring device attached to the 3rd digit of the right hand at a sampling rate of 100 Hz (Finometer, Finapres BV, The Netherlands), to determine vascular sympathetic activity and baroreflex sensitivity.

We quantified vascular sympathetic activity and baroreflex sensitivity by spectral analysis techniques as described previously[120]. In short, recordings were subdivided into data segments of 100 s, overlapping for 50%, and resampled at 5.12 Hz. Each segment was then analyzed with a Fast Fourier Transformation that searches for rhythmic fluctuations in SBP and pulse interval (PI, the reciprocal of HR) with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. Subsequently, the SBP and PI powers were expressed as a function of the frequency. Vascular sympathetic activity (SYMP) was defined as the natural logarithm of the power of the low frequency (LF; 0.04-0.15 Hz) component of the variations in SBP. Baroreflex sensitivity (BRS), which provides information about the changes in heart rate (output) in response to fluctuations in SBP (input), was defined as the LF transfer gain from SBP to PI and expressed in $ms \cdot mmHg^{-1}$.

Venous compliance (VeC) was measured by strain gauge venous occlusion plethysmography with direct intravenous pressure measurement. An intravenous catheter was inserted in an antecubital vein and connected to a pressure transducer system at atrial height. Changes in forearm volume were measured by a mercury-in-silastic strain gauge at 5 cm distal to the antecubital crease. Changes in limb volume were expressed in milliliters per deciliter of limb tissue. A venous collecting cuff was placed 5 cm proximal to the antecubital crease. The pressure cuff was connected to a rapid cuff inflator (Hokanson E20, Denmark) to ensure rapid and accurate filling and deflation of the cuff. Data signals were recorded with a computer system, at a sampling rate of 100 Hz, and stored for further analysis (MIDAC; Biomedical Engineering Department, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands).

Cuff pressure was gradually increased from 0 to 40 mmHg in 60 seconds. Changes in forearm volume and intravenous pressure were recorded. VeC was defined as the ratio of the slope of volume-time curve and the slope of the pressure-time curve:

$$VeC = \frac{\Delta\, volume \,/\, \Delta\, time}{\Delta\, pressure \,/\, \Delta\, time}$$

Only the data of the linear part of this relationship were used, until the increase rate of intravenous pressure and forearm volume diminishes.

Statistical analysis:

Data are presented as medians (interquartile ranges), unless otherwise stated. In all participants, differences between venous compliance, baroreflex sensitivity and vascular sympathetic activity at supine position and the various tilt angles were analyzed using the Wilcoxon Signed Rank test. The responses to head-up tilt in venous compliance, vascular sympathetic activity and baroreflex sensitivity, were quantified by calculating the area-under-the-curve (AUC) and by linear regression analysis (Spearmans' Rho).

Differences between the three plasma volume groups, with respect to basal values and responses to head-up tilt, were analyzed using the Kruskal-Wallis test. When the Kruskal-Wallis test indicated a significant difference, the Mann-Whitney U test was performed to assess differences between 2 separate groups, in which the low plasma volume group served as the reference group.

Some women experienced pre-syncope and could not complete the tilt test. Pre-syncope was defined by symptoms of light-headedness, blurred vision, dizziness and/or nausea. In those women, the response to orthostatic stress, i.e. the AUC and regression analysis, was performed on the data until 40 degrees HUT. For the participants who were able to complete the test, both the response data till 40 degrees and till 60 degrees were analyzed and compared. When no differences were observed, the data till 60 degrees were used.

To identify the independent predicting factors for venous compliance and vascular sympathetic activity in supine position, the venous and autonomic responses to orthostatic

stress, and the susceptibility to pre-syncope, we performed multiple linear regression analysis and multivariate backward stepwise logistic regression analysis, with body mass index, resting mean arterial blood pressure and resting heart rate as co-variables.

A p-value of less than 0.05 was considered as being statistically significant.

Results

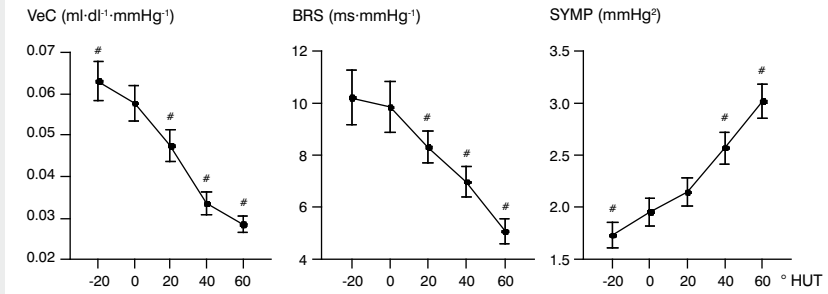
We included 30 normotensive, formerly preeclamptic women with a median age of 31 (27-34) years, body mass index (BMI) of 23 (21-28) $kg \cdot m^{-2}$ and a resting mean arterial pressure of 86 (80-93) mmHg. Fourteen percent was smoking with a median of 13 (6-17) cigarettes/day. As a whole, during positive head-up tilt, venous compliance and baroreflex sensitivity decreased and vascular sympathetic activity increased in all women (Figure 2).

Plasma volume linearly related to venous compliance in supine position ($r=0.64$, $p<0.01$) as shown in Figure 3. The response of venous compliance during the tilt test, as indicated by the AUC as well as the linear regression coefficient of venous compliance and tilt angle, also correlated with plasma volume ($r=0.59$, $p<0.01$, $r=-0.40$, $p=0.03$, respectively).

To further explore the role of plasma volume in venous response capacity, all women were divided into three groups, based on their plasma volume. Plasma volume was 49 (39-52), 56 (54-58) and 62 (59-69) $ml \cdot kg_{LBM}^{-1}$ in the low (LPV), medium (MPV) and high plasma volume (HPV) group, respectively. The three groups did not differ in age, smoking habits and obstetric history, except for lower body mass index (BMI) in the highest plasma volume group as compared to the LPV-group (Table 1). Table 2 shows the hemodynamic and autonomic values in supine position. Blood pressure, vascular sympathetic activity and baroreflex sensitivity were comparable between groups. VeC was 0.044 (0.034-0.054) $ml \cdot dl^{-1} \cdot mmHg^{-1}$ in the LPV-group, which was lower than VeC in the MPV-group (0.054 (0.048-0.081) $ml \cdot dl^{-1} \cdot mmHg^{-1}$, $p=0.04$) and the HPV-group (0.069 (0.049-0.087) $ml \cdot dl^{-1} \cdot mmHg^{-1}$, $p=0.03$).

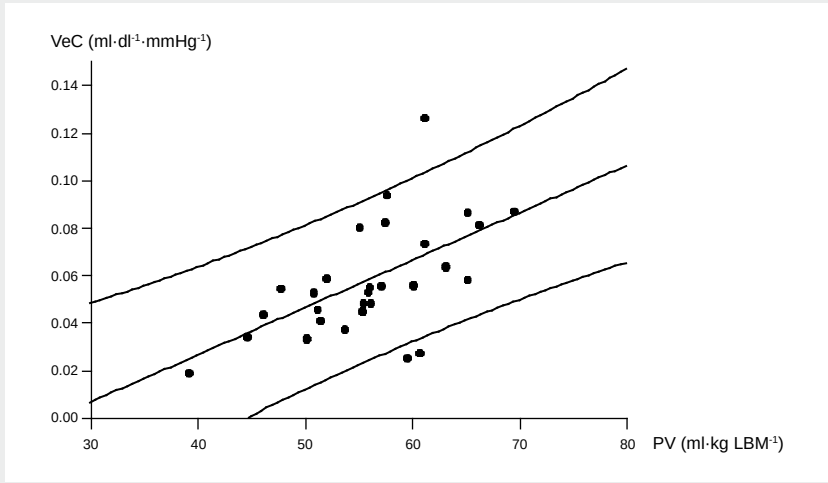
The three groups demonstrated different magnitudes in response of venous compliance during head-up tilt (Figure 4). LPV blunts the total response curve of venous compliance

Figure 2



Venous compliance (VeC), baroreflex sensitivity (BRS) and sympathetic activity (SYMP) in response to head-up tilt (HUT) in all participants. Values are presented as mean ± SEM. # $p<0.05$ as compared to supine position.

Figure 3



Relation between venous compliance (VeC) and plasma volume (PV, $r=0.64$, $p<0.01$) in supine position. Regression line and 90% confidence interval presented.

Table 1 Baseline characteristics of the low, medium and high plasma volume groups.

	Low	Medium	High
Age (years)	32 (29-33)	28 (25-31)	32 (30-36)
BMI (kg.m^{-2})	27 (23-31)	25 (21-27)	21 (20-24)*
Smoking (%)	20%	10%	10%
Onset PE/HELLP (days)	202 (191-229)	200 (180-257)	215 (199-224)
GA at delivery (days)	212 (201-246)	228 (197-269)	234 (215-249)
Birth weight (g)	977 (852-1863)	1015 (583-2230)	1503 (1190-2338)
Birth weight percentile (%)	7.4 (2.5-26.6)	8.4 (1.8-19.3)	16.6 (8.0-39.7)

BMI: body mass index, PE: preeclampsia, HELLP: hemolysis, elevated liver enzymes, low platelets syndrome, GA: gestational age. * $p<0.05$ as compared to the low plasma volume group.

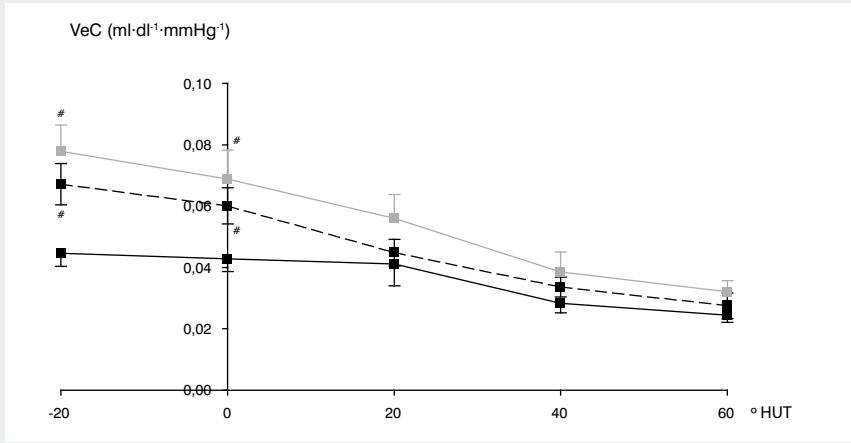
Table 2 Baseline hemodynamic values of the low, medium and high plasma volume groups.

	Low	Medium	High
SBP (mmHg)	118 (112-134)	122 (106-128)	115 (108-125)
DBP (mmHg)	71 (62-76)	72 (64-76)	68 (66-72)
HR (bpm)	72 (67-84)	72 (63-76)	69 (65-77)
VeC (ml·dl^{-1}·mmHg^{-1})	0.04 (0.03-0.05)	0.05 (0.05-0.08)*	0.07 (0.05-0.09)*
SYMP (mmHg^{2})	1.8 (1.4-3.0)	2.0 (1.6-2.4)	1.9 (1.3-2.4)
BRS (ms·mmHg^{-1})	8.8 (4.2-13.3)	9.5 (7.8-13.6)	8.9 (7.9-12.9)

SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, VeC: venous compliance, SYMP: vascular sympathetic activity, LF/HF ratio: cardiac autonomic balance, BRS: baroreflex sensitivity.
* $p<0.05$ as compared to the low plasma volume group.

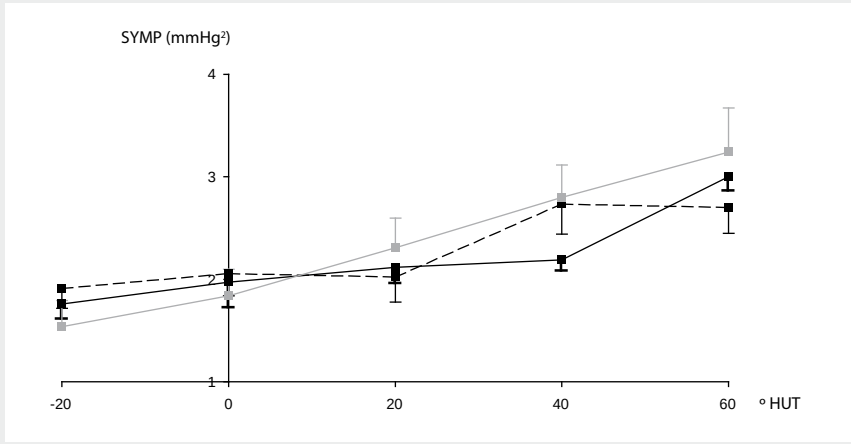
during HUT, as evaluated by the AUC and regression analysis. In the MPV-group, there was a larger response (loss of venous compliance: -0.68 (-0.88- -0.36) $\cdot 10^{-3}$ ml·dl^{-1}·mmHg^{-1}.^{o}HUT^{-1}) as well as in the HPV-group (-0.67 (-0.77- -0.49) $\cdot 10^{-3}$ ml·dl^{-1}·mmHg^{-1}.^{o}HUT^{-1}) as compared to the LPV-group (-0.28 (-0.49- -0.17) $\cdot 10^{-3}$ ml·dl^{-1}·mmHg^{-1}.^{o}HUT^{-1} ($p=0.03$ and $p=0.04$ respectively). At 60 degrees head-up tilt, VeC was comparable in all groups (0.027 (0.020-0.029), 0.024 (0.019-0.039) and 0.029 (0.024-0.037) ml·dl^{-1}·mmHg^{-1} in the LPV, MPV and HPV-group, respectively).

Figure 4



Mean (± SEM) venous compliance (VeC) in response to head-up tilt (HUT) in the low plasma volume group (**–■–**), medium plasma volume group (**--■--**) and high plasma volume group (–■–). * $p<0.05$ as compared to the low plasma volume group.

Figure 5



Mean (± SEM) sympathetic activity (SYMP) in response to head-up tilt (HUT) in the low plasma volume group (**–■–**), medium plasma volume group (**--■--**)) and high plasma volume group (–■–). There were no differences between groups, at each rotational step.

The change in vascular sympathetic activity in the three groups is presented in Figure 5. The response pattern to HUT as indicated by the (individual) regression lines is lower in women with LPV (rise in sympathetic activity =0.009 (0.002-0.014) $mmHg^{2}{\cdot}^{o}HUT^{-1}$, Figure 6) as compared to the HPV-group (0.022 (0.016-0.029) $mmHg^{2}{\cdot}^{o}HUT^{-1}$, $p<0.01$). Changes in baroreflex sensitivity during head-up tilt were comparable in the three PV-groups.

Nine women (30%) experienced pre-syncope at 60 degrees head-up tilt. Women with pre-syncope had lower plasma volume than women who were able to withstand the orthostatic stress (54 (48-59) versus 57 (52-62) $ml{\cdot}kg_{LBM}^{-1}$, $p=0.03$).). Venous and autonomic responses were similar in those with and without pre-syncope. Multivariate binary regression analysis on the occurrence of pre-syncope in relation to plasma volume, BMI, mean arterial blood pressure and heart rate as co-variables pointed out only plasma volume to be an independent predicting factor for pre-syncope ($p=0.04$).

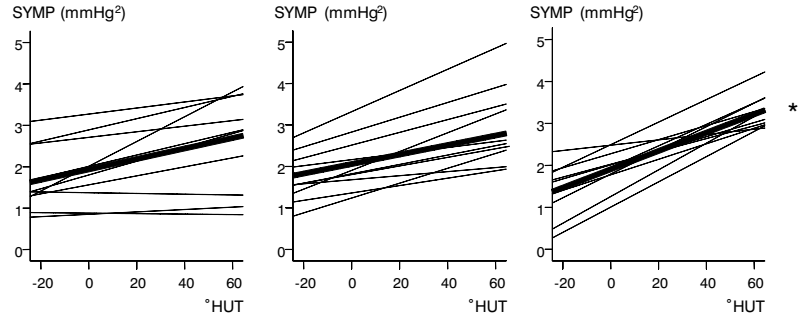
To evaluate possible effects of body posture, blood pressure and heart rate on venous functioning, we applied multiple linear regression analyses. At these analyses, both venous compliance in supine position as well as the venous response to HUT was not affected by these variables. BMI also did not influence resting vascular sympathetic activity ($p=0.41$). Both BMI ($p=0.03$) and plasma volume ($p<0.01$) were independent predicting factors in the response of vascular sympathetic activity during head-up tilt.

Discussion

In non-pregnant formerly preeclamptic women, plasma volume is positively related to resting venous compliance and to the magnitude of the venous response to head-up tilt. The capacity to lower venous compliance in response to orthostatic stress was attenuated in those with low plasma volume. Moreover, women with low plasma volume exhibited a smaller rise in vascular sympathetic activity during the tilt test.

To our knowledge, the venous responses to slowly increasing orthostatic strain have never been studied before in formerly preeclamptic women. We previously reported a continuously higher heart rate and reduced tolerance to orthostatic stress in formerly preeclamptic women with low plasma volume as compared to those with normal

Figure 6



Individual (thin) and mean (thick) regression lines of the response of (vascular) sympathetic activity (SYMP) to head-up tilt (HUT) in the low (left panel), medium (middle panel) and high (right panel) plasma volume group. * $p<0.05$ as compared to low plasma volume group.

plasma volume, suggesting a limited ability to sufficiently mobilize unstressed volume[6].

The results of the present study support the view that formerly preeclamptic women with low plasma volume have indeed a reduced venous reserve capacity. Reduced venous reserve capacity may originate from small venous dimensions, which is in line with shallow venous development as part of the 'fetal origin of disease' complex within the Barker hypothesis[194]. Second, reduced venous compliance may also result from altered venous wall properties. These may follow from changes in vascular matrix or muscular contraction as a result of secondary sympathetic overactivity, such as in the metabolic syndrome. We were unable to demonstrate resting autonomic differences between plasma volume groups, even though other studies on similar populations reported an increased sympathetic tone in formerly preeclamptic women with chronic low plasma volume[5;6].

Autonomic dysfunction could also underlie the attenuated venous response in women with low plasma volume. Veins are richly innervated by sympathetic nerves, which are deeply attached to the smooth muscle cells of the venous wall, leaving them highly sensitive to sympathetic modulation[12;13]. The autonomic regulation of the venous system is primarily dominated by the baroreceptor reflex system. Our results may indicate failure of the baroreflex control on vascular sympathetic activity, resulting in an inability

to sufficiently constrict the veins at increased tilt. We did not observe differences in baroreflex sensitivity between groups, leaving the possibility of an abnormality in the pathway to the activation of vascular sympathetic activity or the signal transduction to the venous vessels[198].

It is tempting to correlate the venous responses to the changes in vascular sympathetic activity. However, caution needs to be taken when interpreting the relation between spectral results and venous functioning. Autonomic function, as assessed by spectral analysis, is based on the fluctuations in systolic blood pressure and heart rate. Although arterial and venous function is coupled and act in concert with each other[132], our sympathetic data are, predominantly, a measure of the arterial and cardiac sympathetic control and cannot be translated to a measure of sympathetic activity on veins. Spectral analysis on fluctuations in venous pressure or interventions altering venous sympathetic activity (α-andrenoceptor antagonists or ganglionic blockers)[132] might be useful in future research to differentiate between the possible abnormalities in the sympathetic control of the venous system.

The cause of pre-pregnant low plasma volume is currently unknown. These women are not chronically vascular underfilled since compensatory neuro-humoral changes such as elevated renin, angiotensin, and aldosterone levels are lacking and baseline hemodynamic values are quite similar to their normal plasma volume counterparts[4]. In addition, reports on renal hemodynamics and post-occlusive forearm blood flow suggest that low plasma volume is not supposed to be caused by defective renal or endothelial function[4;7;8;199]. Therefore, studies on this phenotype are different from those inducing low plasma volumes by head-down bed rest, diuretics, or space flight.

In this study, we were interested in the role of plasma volume in the venous and autonomic response to head-up tilt in formerly preeclamptic women. We did not include a group of women with an uncomplicated obstetric history. We assume that the response of healthy parous controls to head-up tilt will mimic our results of the formerly preeclamptic women with higher plasma volumes, as first, the definition of low plasma volume is based on the values of those with an uncomplicated obstetric history and second our previous findings indicate similarities in circulatory responses to alterations in vascular resistance between healthy parous controls and formerly preeclamptic women with normal plasma volume[4;11;188].

Body composition may have affected our findings. In order to compensate for BMI, we adjusted plasma volume for lean body mass, as fat tissue is poorly vascularised[200-202]. Nonetheless, BMI still could have confounded our data as obesity is known to alter the cardiovascular autonomic control[203;204]. To explore a possible role for BMI, we performed multiple regression analyses. In these analyses BMI did not affect the venous response curve to orthostatic stress, but related, in combination with plasma, to the change in vascular sympathetic activity during head-up tilt. Therefore, body composition is, at least partly, responsible for our observations on sympathetic activity.

Venous compliance was measured at the forearm instead of the calf, as used in most studies. Loss of (intravascular) plasma volume occurs during head-up tilt due to increased fluid filtration, affecting tissue pressure[150;152]. We measured venous compliance at the forearm to minimize muscle contraction and capillary filtration, as both affect venous compliance[119;147;148]. Even so, the splanchnic veins are thought to play the most important role in restoring venous return as these vessels contain most venous blood. Active distribution of venous volume, by reducing venous compliance in the remaining part of the venous system, accounts for only 25% of the total blood transfer[205]. In the present study, we intended to qualify this response, but it may be possible that women with low plasma volume especially exhibit reduced ability to mobilize splanchnic blood.

Another limitation of the study is that the level of physical fitness was not determined prior to the study. The latter is known to be correlated with plasma volume, sympathetic tone and venous compliance[133;195;206;207]. Although we have no reason to suppose a difference in physical condition between groups, reduced physical condition could have affected the number of women with low plasma volume or altered resting vascular sympathetic activity and venous compliance and their response to head-up tilt.

In conclusion, plasma volume relates to resting venous compliance and the magnitude of the response during head-up tilt in formerly preeclamptic women. Women with low plasma volume exhibit reduced venous response capacity, which may originate from reduced venous dimensions and/or altered sympathetic control over the venous circulation.

Chapter 7

Blunted autonomic response to volume expansion in formerly preeclamptic women with low plasma volume

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Abstract

Objective: We hypothesize that low plasma volume (PV) in normotensive formerly-preeclamptic women reflects reduced venous storage capacity. To test this hypothesis, we compared circulatory and autonomic responses to acute volume loading between women with low (LPV) and those with normal PV (NPV).

Methods: In 24 normotensive formerly-preeclamptic women at least 6 months post partum, we administered in 30 minutes 500 ml of iso-oncotic fluid by constant i.v. infusion, while recording changes in heart rate (HR), blood pressure (BP), cardiac output (CO), and measuring active plasma renin (APRC) and α-atrial natriuretic peptide (α-ANP) concentrations. We estimated arterial sympathetic control (SYMP), cardiac autonomic regulatory balance and baroreflex sensitivity (BRS), using spectral analysis. Inter- and intragroup changes were analyzed non-parametrically. Results: Seventeen women (71%) had LPV and 7 (29%) had NPV. PV expansion induced comparable changes in BP, HR, BRS and APRC in LPV and NPV. CO and α-ANP increased in LPV, but not in NPV. Volume expansion reduced SYMP (from 2.41 to 1.76 $mmHg^2$, $p=0.03$) in NPV, but not in LPV (2.72 to 2.48 $mmHg^2$, $p>0.05$).

Conclusion: The sympatho-inhibitory response to volume expansion is diminished in LPV, which suggests that cardiovascular reflex function is impaired. We speculate that this defect contributes to circulatory maladaptation to pregnancy, sympathetic dominance and the development of gestational hypertensive disease.

Introduction

Women with a history of preeclampsia are at elevated risk to develop cardiovascular disease in later life, as indicated by an increased prevalence of hypertension, ischemic heart disease and stroke[208]. These associations suggest that hemodynamic abnormalities in formerly preeclamptic women and in patients with cardiovascular diseases share similar characteristics.

About one out of four normotensive formerly-preeclamptic women have low plasma volume (LPV) at least 6 months postpartum[4;8]. The autonomic control of the circulation in these women is characterized by a sympathetic predominance and a reduced venous compliance[5;6;10]. As a consequence, these women have a reduced ability to raise stroke volume in response to modest physical exercise[114]. In addition, they also respond differently to the pregnancy-induced fall in afterload, when compared to their counter-parts with a pre-pregnant normal plasma volume (NPV). These aberrant responses to pregnancy include a shallow increase in plasma volume, a limited increase in venous and arterial compliance and a paradoxical rise in α-atrial natriuretic peptide (α-ANP)[10;11]. It is likely that these abnormal responses contribute to the 3-times higher chance to develop recurrent gestational hyperten-sive disease as compared to their counterparts with pre-pregnant NPV[8]. These observations suggest that LPV identifies women with a reduced venous storage capacity, with an inadequate ability to accommodate extra volume in pregnancy[18]. We speculate that the autonomic cardiovascular abnormalities in formerly preeclamptic women, such as reduced baroreflex sensitivity, resemble those observed in early stages of hypertension[27;146;209;210].

Human and animal studies have demonstrated that volume expansion causes inhibition of the sympathetic system via activation of the (arterial) baroreceptors and the cardiopulmonary low-pressure receptors[26;211-215]. In hypertensive subjects, the sympatho-inhibitory response to volume expansion is blunted and even leads to venous overfill[216]. The mechanism underlying the blunted sympatho-inhibitory response might be an inadequate autonomic adaptation possibly due to impaired baroreceptor control of peripheral vasomotor tone[213;217;218]. We hypothesize that in formerly-preeclamptic women with LPV, the buffering capacity of the venous compartment is limited and that the autonomic response to volume loading is similarly blunted. To this end, we compared the

autonomic and circulatory responses to volume expansion of formerly preeclamptic women with LPV and those of formerly preeclamptic women with NPV.

Methods

Subjects

Twenty-four normotensive, non-pregnant women with a history of pre-eclampsia with or without HELLP-syndrome (hemolysis, elevated liver enzymes, low platelets) were included in the study. All women were at least 6 months postpartum, not breastfeeding and not taking any medication.

Preeclampsia and HELLP-syndrome were defined according to the criteria of the International Society on the Study of Hypertension in Pregnancy (ISSHP)[190]. Informed consent was obtained from all participants and the study was approved by the Institutional Review Board.

Experimental design

All measurements were performed in the follicular phase of the menstrual cycle to minimize hormonal influence. All participants refrained from smoking and alcohol consumption at least 10 hours before the start of the experiments. Prior to volume loading, all women underwent plasma volume measurement and echocardiographic evaluation. Researchers were blinded to the plasma volume and echocardiographic results at the time of the infusion period and data analysis.

All measurements were performed under controlled environmental conditions, in a quiet and partially darkened room with an ambient temperature of 23°C, with subjects comfortably lying in supine position. Participants remained in this position for 30 minutes before the volume expansion test was started.

Before and after the infusion period, blood samples were collected for measurement of α-ANP ($ng \cdot L^{-1}$) and active plasma renin concentration (APRC, $mU \cdot L^{-1}$). The biochemical assays for α-ANP and APRC have been described in detail before[11]. We intravenously administered 500 ml iso-oncotic fluid (Voluven®) of a temperature of 37°C, at a constant rate of 16.7 $ml \cdot min^{-1}$.

Measurements

Plasma volume (PV) was measured using the Dextran dilution method[219] (coefficient of variation 5%) and expressed in milliliters per kilogram lean body mass (LBM). For this purpose, we calculated LBM as weight minus body fat mass. Body fat mass (BF) was estimated by the formula as described by Deurenberg, *et al*[220]:

$$BF = ((1.2 * BMI) + (0.23 * age) - (10.8 * gender) - 5.4) * \frac{weight}{100}$$

where body mass index (BMI) is expressed in $kg \cdot m^{-2}$, age in years, gender in points (1 for male, 0 for female) and weight in kilograms. We considered a PV < 48 $ml \cdot kg_{LBM}^{-1}$ as low plasma volume, corresponding with two standard deviations below the mean reported for healthy parous controls[4].

Baseline echocardiographic indices were measured by an experienced cardiologist, who measured the left atrial diameter, the left ventricular outflow tract velocity integral and diameter as previously been described in detail[19;221]. Measurements were performed in the left lateral position, using a cross-sectional phased array echocardiographic Doppler system (Vivid 7, General Electric, Horten, Norway). Heart rate was derived from the RR-interval of the ECG measured during the echo Doppler measurements. Stroke volume (SV) was calculated by multiplying the left ventricular outflow tract velocity integral and the left ventricular outflow tract diameter. Cardiac output (CO) was calculated as SV times HR and total peripheral vascular resistance (TPVR) as eighty times the mean arterial pressure (MAP), divided by the cardiac output (80*MAP/CO). The index values were calculated by dividing the SV, CO and TPVR by the body surface area, as determined by the method described by Du Bois and Du Bois[191].

During the infusion period, we recorded changes in HR and BP by a finger arterial BP-monitoring device attached to the 3rd digit of the right hand at a sampling rate of 100 Hz (Portapress, Finapres BV, The Netherlands). Data were obtained during five-minutes intervals, starting before and 5, 15 and 25 minutes after the start of the infusion period. Relative changes in SV and CO were determined by continuous beat-to-beat pulse contour analysis from the Portapress. The changes in SV and CO were transformed to absolute values, by relating them to the values measured by echocardiography at rest prior to the test.

Table 1 Demographic and baseline hemodynamic characteristics of women with normal plasma volume (NPV) and low plasma volume (LPV).

	NPV	LPV	p value
Number of women	7	17	
Age (yr)	31 (29-36)	29 (27-32)	ns
BMI ($kg \cdot m^{-2}$)	23 (21-24)	27 (24-30)	<0.05
Onset PE/HELLP (wk)	32 (31-35)	30 (25-34)	ns
Mean arterial blood pressure (mmHg)	89 (76-93)	88 (81-94)	ns
Left atrial diameter index ($mm \cdot m^{-2}$)	20 (19-21)	21 (18-22)	ns
Stroke volume index ($ml \cdot m^{-2}$)	46 (38-46)	43 (39-51)	ns
Heart rate (bpm)	71 (63-78)	68 (66-74)	ns
Cardiac index ($l \cdot min^{-1} \cdot m^{-2}$)	2.9 (2.5-3.1)	2.8 (2.6-3.6)	ns
TPVR index ($dyne \cdot s \cdot cm^{-5} \cdot m^{-2}$)	776 (659-1088)	679 (598-802)	ns
Volume loading induced rise in PV (%)	22 (21-24)	24 (21-25)	ns

Values presented as median (interquartile ranges).
ns: not significant, BMI: body mass index, PE: pre-eclampsia, HELLP: haemolysis, elevated liver enzymes, low platelets syndrome, TPVR: total peripheral vascular resistance, PV: plasma volume

We quantified autonomic activity and baroreflex sensitivity from the spontaneous fluctuations in HR and BP by spectral analysis technique[120]. The recordings were subdivided into data segments of 100 s, overlapping for 50%, and resampled at 5.12 Hz. Each segment was then analyzed with a Fast Fourier Transformation that searches for rhythmic fluctuations in systolic blood pressure (SBP) and pulse interval (PI) with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. Subsequently, the SBP and PI powers were expressed as a function of the frequency. We defined (vascular) sympathetic activity (SYMP) as the natural logarithm of power of the low frequency component (0.04-0.15 Hz) of the variations in SBP. The ratio of absolute low (LF) and high frequency (HF) powers of the PI was assumed to represent the cardiac autonomic balance between the sympathetic and vagal system. Baroreflex sensitivity (BRS, $ms \cdot mmHg^{-1}$), which provides information about the changes in HR (output) in response to fluctuations in SBP (input), was defined as the (LF) transfer gain from SBP to PI.

Statistical analysis

Data are presented as median (interquartile ranges), unless otherwise stated. Differences between groups, in basal values and responses to volume expansion, were analyzed using the Mann-Whitney-U test. For each group, differences in the response to volume loading were analyzed by the Wilcoxon Signed Rank Test. As demographic variables, with possible confounding effects on results during volume loading, might differ between groups, we performed regression analyses on the end-values of mean arterial pressure, heart rate, cardiac output, α-ANP, cardiac autonomic balance, vascular sympathetic activity and baroreflex sensitivity, with their respective baseline values as well as plasma volume as possibly predicting variables. Spearman correlation was used to assess correlations between continuous variables. We considered a p-value below 0.05 as statistically significant.

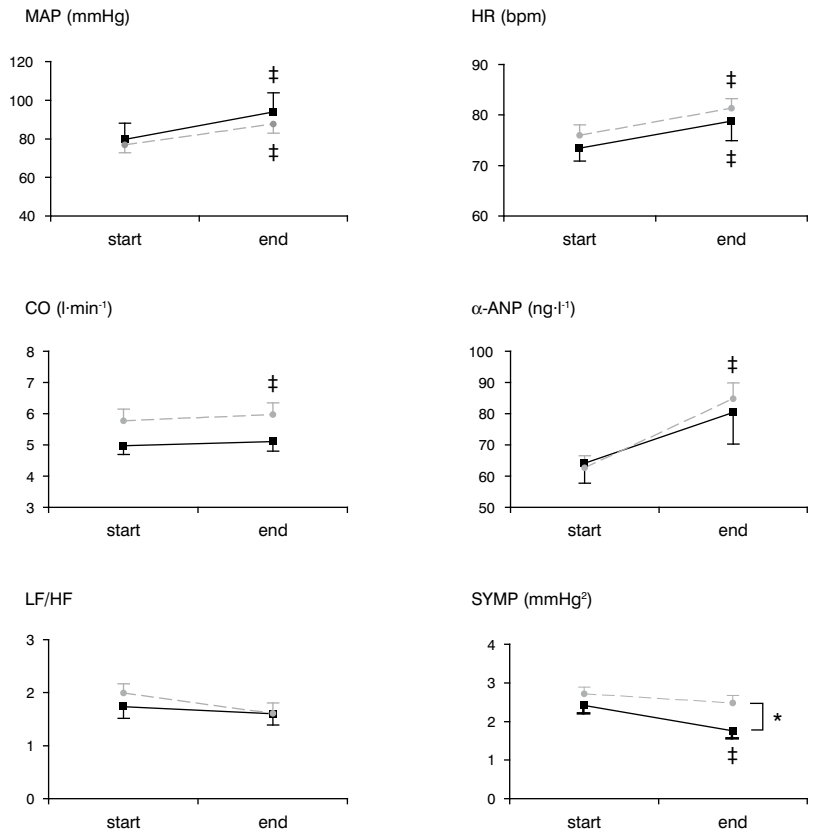
Results

The 24 participants consisted of 17 (71%) formerly-preeclamptic women with LPV and 7 (29%) with NPV. The median plasma volume in the LPV and NPV subgroups were 43 (41-45) $ml \cdot kg_{LBM}^{-1}$ and 50 (48-50) $ml \cdot kg_{LBM}^{-1}$ ($p<0.01$), respectively. Table 1 lists the demographic and basal hemodynamic characteristics of the two subgroups. The LPV subgroup had a higher BMI. Mean arterial pressure, cardiac index, total peripheral vascular resistance index and left atrial diameter index were comparable in the two subgroups.

Obstetric history did not differ between the NPV-and LPV-women with respect to gestational age at onset of preeclampsia and the occurrence of the HELLP-syndrome.

Figure 1 depicts the hemodynamic and autonomic changes at the start and end of the infusion period. In the NPV and LPV subgroups, volume loading induced comparable increases in mean arterial pressure (20% and 14%, respectively) and heart rate (7% and 8%, respectively). In contrast, volume loading induced a consistent rise in cardiac output, α-ANP and BRS only in women with LPV. In women with NPV, a consistent fall in SYMP (-30%) was observed. Finally, at the end of the volume loading SYMP was 44% higher in LPV than in NPV. In both subgroups

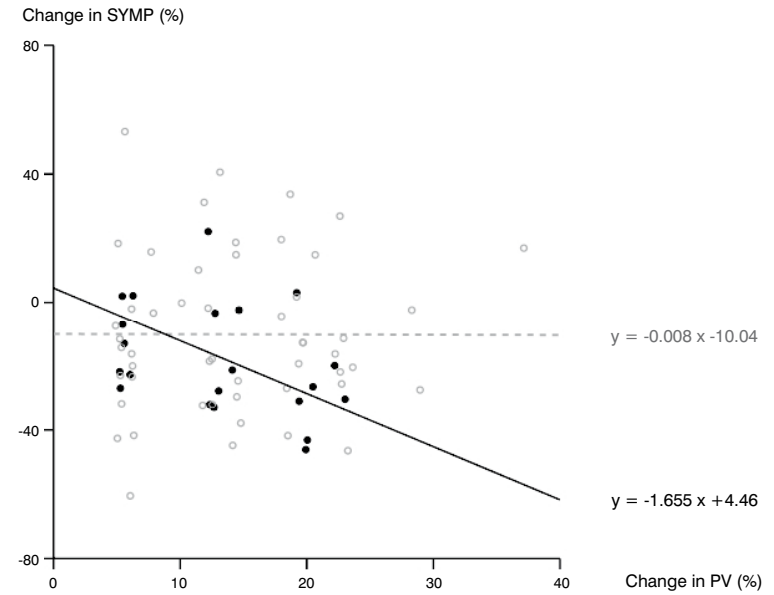
Figure 1



The response to volume expansion in mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), α-atrial natriuretic peptide (α-ANP), autonomic balance (LF/HF) and sympathetic activity (SYMP) in 7 non-pregnant women with normal plasma volume (–■–) and 17 non-pregnant women with low plasma volume (--●--).
‡ $p<0.05$ compared to start value, * $p<0.05$ between groups.

volume expansion decreased APRC levels to a similar extent. The cardiac autonomic balance (LF/HF ratio) remained unaltered in response to volume expansion in both groups.

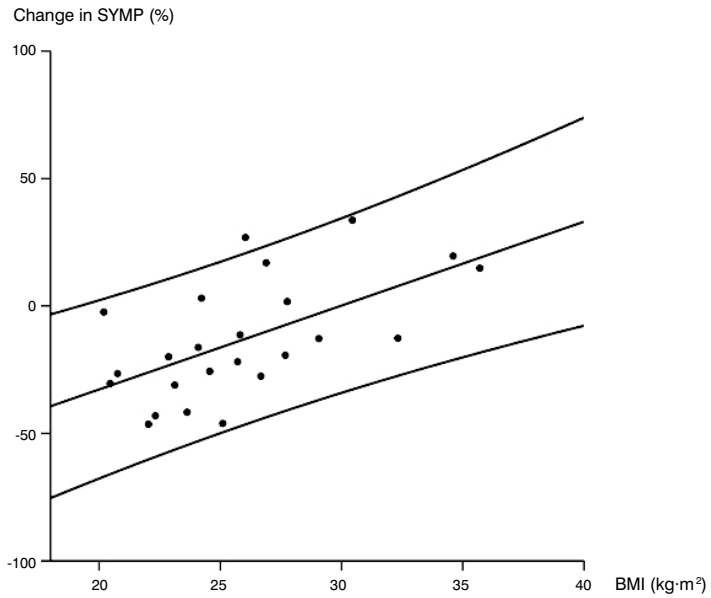
Figure 2



Percentage change in sympathetic activity (SYMP) in response to the volume load relative to the original plasma volume (PV), 5, 15 and 25 minutes after the start of infusion. Negative correlation in 7 women with normal plasma volume (●, black line, $r=0.49$, $p=0.004$), but not in 17 women with low plasma volume (○, dotted grey line, $r=0.0$, $p=0.984$).

Volume loading induced a similar percentage rise in PV in the NPV- and LPV-groups (Table 1). To make sure that the induced differences in hemodynamics in both groups did not result from the relative differences in volume load, we also correlated the responses of mean arterial blood pressure, heart rate, cardiac output, SYMP and the percentage rise in α-ANP (measured after volume loading) with the percentage rise in PV induced by volume loading after 5, 15 and 25 minutes. The relative change in PV was expressed as the percentage rise in PV relative to the initial PV at 5, 15 and 25 minutes. In both subgroups, the percentage change in PV was positively correlated to the changes in MAP and HR. In the LPV-group, but not in the NPV-group, there was a significant correlation between the relative PV increase and that in CO ($r=0.37$, $p<0.01$) as well as the relative PV increase and that in α-ANP ($r=0.72$, $p<0.01$). Concomitant changes in SYMP were

Figure 3



Correlation between percentage change in vascular sympathetic activity (SYMP) in response to the volume load and body mass index (BMI). Lines represent mean linear regression (r=0.62, p<0.01) and 90% confidence interval.

inconsistent in LPV-women. In contrast, in the NPV-subgroup, the percentage rise in CO and that in α-ANP did not correlate with the percentage rise in PV, but SYMP reduced gradually in response to expanding PV (r=-0.49, p<0.01, Figure 2).

As body mass index (BMI) may affect the observations, we reanalyzed our data and correlated the hemodynamic and autonomic changes induced by volume loading to BMI. We found that a high BMI blunted the sympatho-inhibitory response (r=0.62, p<0.01, Figure 3).

Moreover, a higher BMI related to a larger decrease in TPVR (r=-0.54, p=0.01) and a larger increase in CO (r=0.42, p=0.04) during volume expansion, but a lower increase in systolic BP (r=-0.49, p=0.02; data not shown). Differences in baseline values

between groups may be a consequence of differences in BMI. Therefore, we performed regression analyses on the end-values of all hemodynamic and autonomic variables, with their respective baseline values as well as plasma volume as possibly predicting variables. In these analyses, baseline values for heart rate (p<0.01), cardiac output (p<0.01), α-ANP (p<0.01), cardiac autonomic balance (p=0.04) and baroreflex sensitivity (p<0.01) all independently predict their respective end-values, whereas plasma volume did not. The end-values of mean arterial pressure and vascular sympathetic activity were independently predicted by both their respective baseline value (p<0.01 respectively p=0.01) *and* plasma volume status (both p=0.03).

Discussion

In this study, we tested the hypothesis that the buffering capacity of the venous compartment is limited in formerly-preeclamptic women with low plasma volume and that the autonomic response to volume loading is altered.

In women with normal plasma volume, the hemodynamic responses to volume loading were as expected and in line with previous data[26;214;215]. Blood pressure and heart rate increased, the latter most likely due to a direct effect of the increased atrial volume to stretch the sinus node thus activating the Bainbridge reflex. The Bainbridge reflex is a tachycardic response to a rise in central venous pressure, stimulated by the mechanoreceptors located at the junction of the right atrium and caval veins or at the junctions of the pulmonary veins and the left atrium. This reflex prevents gathering of the blood in the veins, atria and pulmonary circulation[26]. Because cardiac autonomic balance (LF/HF ratio) did not change upon volume loading we suggest that the Bainbridge reflex may have masked the expected cardiac sympatho-inhibition in response to the activation of the low- and high-pressure baroreceptors (i.e. increase in arterial blood pressure). In contrast, the vascular sympathetic activity decreased in response to volume loading as expected in women with normal plasma volume, suggesting a differential sympathetic control to volume loading.

Interestingly, in women with low plasma volume the decrease in vascular sympathetic activity was absent, while increments in blood pressure and heart rate were comparable to those observed in NPV. The most appealing explanation for the

blunted sympatho-inhibitory response to volume loading in women with LPV would be that the women were actually underfilled and that volume loading has corrected the defect. However, in that case baseline hemodynamics as well as baseline values for α-ANP and renin should have been different between the LPV and NPV group, which we did not observe in this study. The rise in both α-ANP and cardiac output in the LPV-subgroup supports the view that these women were unable to accommodate extra volume in their venous compartment and that volume loading induced a state of relative circulatory overfill.

We speculate that the blunted inhibition of SYMP in the LPV-subgroup may be either due to a reduced cardiovascular reflex function or to the effects of α-ANP, or both. Decreased baroreflex control of vascular sympathetic activity in the LPV subgroup might contribute to the abnormal responses to volume loading. Charkoudian, et al observed reduced sensitivity of baroreflex control of muscle sympathetic nerve activity (MSNA), but normal baroreflex functioning of the heart, at increased central venous pressure[215]. We did not measure central venous pressure due to the minimal invasive design of this study, but as we expect our LPV-subgroup to exhibit low venous capacitance, volume loading will induce a rise in central venous pressure. Similar to hypertensive subjects[217], resetting and/or defective baroreflex control of the vascular sympathetic activity might also occur in our study-group giving rise to the lack of sympatho-inhibition in women with LPV. Nonetheless, we did not observe a difference in cardiac autonomic balance between both groups indicating an intact baroreflex control of heart rate in women with LPV. The apparently altered baroreflex control of the vasculature has never been described in these women and therefore requires confirmation by direct MSNA measurements.

The effects α-ANP on the circulation are complex. This hormone promotes vasodilatation, natriuresis, capillary leakage, and inhibits the renin-angiotensin system[67]. Its effect on autonomic regulation seems time and dose dependent. Studies on α-ANP infusions demonstrated both an initial activation[222-226] followed by inhibition[223;227-230] of the sympathetic nervous system with prolonged infusion. Our results in LPV subjects could reflect the short-term α-ANP effects, with transient sympatho-mimetic effect of α-ANP, being opposed by the normal sympatho-inhibitory response to volume loading.

In addition, we separately analyzed the impact of BMI on our results and observed a correlation between BMI and the volume loading induced changes in sympathetic activity, cardiac output, systolic blood pressure and total peripheral resistance. Indexation for body mass is necessary to enable comparison of plasma volume between subjects, as body mass index and plasma volume are positively related[231]. We adjusted plasma volume for lean body mass, because fat tissue is poorly vascularised. At increasing body mass index, blood volume does not rise proportionally to the increase in body weight[200-202;232;233]. By others, plasma volume per kilogram lean body mass is advocated to be a valuable adjustment[200-202] and is frequently used in studies on this topic. In both obese and non-obese formerly preeclamptic women plasma volume per kilogram lean body mass is lower as compared to healthy women with a normal obstetric history[4]. In our study, plasma volume did not turn out to be an independent predicting variable for the end-values of α-ANP or cardiac output, but the end-value for vascular sympathetic activity was both predicted by its baseline value as plasma volume status. In addition, regression analysis on the relative changes in plasma volume on the one hand, and autonomic and hemodynamic changes on the other hand in time, showed that in women with low indexed plasma volume the sympatho-inhibitory effect was blunted. Still, BMI seems to be one of the strongest factors negatively affecting venous reserve capacity. BMI could have confounded our data as obesity is known to alter the cardiovascular autonomic control[203;204], although the mechanisms remains unclear. We assume that a higher BMI blunts the hemodynamic and autonomic control via a reduced venous reserve capacity. The independent predicting value of both its baseline value as plasma volume status in the regression analysis on the end-value of vascular sympathetic activity supports this view. Insulin resistance, as commonly present in obesity, is related to sympathetic overactivity. In addition, the normal potency of insulin as a venodilator reduces with increasing body mass[234]. Finally, obese subjects have reduced venous distensibility, either due to structural or functional factors, such as high local nonesterified fatty acid levels[235]. Angiotensin-II may contribute to a reduced venous reserve capacity in women with high body mass index. A larger study is needed to explore to what extend venous reserve capacity and BMI contribute, independent or in concert, to the hemodynamic and autonomic response to volume loading.

A shortcoming of this study is that we did not address the potential role of vasopressin in the autonomic control. Vasopressin is known to augment the

sympatho-inhibitory responses, also to volume loading in rats[212]. We do not know whether vasopressin levels differ between LPV and NPV women. Theoretically, one would expect volume loading to induce a larger fall in plasma vasopressin in LPV than in NPV, because of the larger rise in central venous pressure. If so, this effect could have contributed to the blunted sympatho-inhibitory response in LPV. Obviously, this concept needs to be confirmed experimentally.

In our study we did not determine urinary output. Theoretically, a larger rise in α-ANP in women with LPV would be associated with more natriuresis and thus, more volume dissipation. That is to say, the same 500 ml volume loading may have led to less PV expansion in LPV than in NPV women. However, correlations between various hemodynamic variables at 5, 15 and 25 minutes and the increase in PV relative to the initial value results in similar findings, which suggests that lagtime between volume expansion and secondary volume dissipation was sufficiently long to prevent methodology-induced differences in intergroup responses. Therefore, information on urinary output would have added little to the analysis of our data.

In conclusion, a preconceptional low plasma volume blunts the normal sympatho-inhibitory response to volume expansion, as seen in women with NPV. Early-pregnancy does not seem to alter this response. We speculate that the absent sympatho-inhibitory response to volume expansion may contribute to circulatory maladaptation to pregnancy, giving rise to sympathetic dominance and the development of gestational hypertensive disease.

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Chapter 8

Exercise-induced changes in venous vascular function in non-pregnant formerly pre-eclamptic women

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Abstract

Objective: Formerly preeclamptic women with low plasma volume (PV) are at increased risk for recurrent gestational hypertensive disease. We hypothesized that a 4-weeks cycling training in formerly preeclamptic women improves (venous) hemodynamic function.

Methods: In nine formerly preeclamptic women, we examined physical fitness and hemodynamic function, before and after the training. We assessed blood pressure (BP), heart rate (HR), cardiac output (CO), PV and calf and forearm venous compliance (VeC).

Results: After the training, baseline BP and CO remained unchanged, but resting HR decreased (-7%, p=0.02). PV was 8% higher after training (p=0.01). Calf VeC increased (+18%, p=0.02), but not forearm VeC (+14%, p=0.09).

Conclusion: Cycling training improves venous vascular function in formerly preeclamptic women. The decreased resting HR and improvement of VeC suggests reduced sympathetic activity. These rapid exercise-induced changes may improve maternal vascular adaptation in early-pregnancy and with it the risk on (recurrent) gestational hypertensive disease.

Introduction

Preeclampsia complicates almost 8% of pregnancies and is a major cause of maternal morbidity and mortality worldwide[2;236]. It is associated with preexistent vascular, metabolic and clotting abnormalities[2;4;237]. Formerly preeclamptic women are at increased risk for hypertension, cardiovascular disease, venous thromboembolism and stroke in later life[208;238].

After gestation, the majority of normotensive formerly preeclamptic women exhibit subnormal plasma volume, a characteristic associated with reduced venous capacitance and elevated sympathetic tone[5-7]. This condition results in predisposition to recurrent gestational hypertensive disease and fetal growth restriction in a subsequent pregnancy[8;239]. In healthy pregnancy, plasma volume increases in response to the drop in peripheral vascular resistance to maintain blood pressure. This plasma volume expansion, necessary to meet the increased arterial demands of advanced pregnancy, can only be accommodated when venous compliance increases as well. Subnormal plasma volume prior to pregnancy hampers normal venous adaptation and plasma volume expansion in the first weeks of gestation[11]. Interventions that improve venous capacitance may contribute to lowering the risk for recurrent gestational hypertensive disease.

Physical activity is associated with a reduced risk of preeclampsia and cardiovascular disease[245-249]. In sedentary and moderately active subjects, exercise training increases plasma volume[243;266] and venous compliance[133;241;267]. Possibly, exercise-induced improvements in plasma volume and venous compliance may contribute to the reduced risk of recurrent gestational hypertensive disease. However, no study has examined the effects of exercise training on these variables in (formerly) preeclamptic women. Therefore, the aim of this study was to examine the effect of a four-weeks training program on hemodynamic function in non-pregnant formerly preeclamptic women. We hypothesize that exercise training in these women improves non-pregnant circulatory variables known to interfere with maternal vascular adaptation in early pregnancy.

Methods

Subjects

Nine non-pregnant women, with a history of preeclampsia were included in this study. Women were recruited from the obstetric outpatient clinic at follow up. Preeclampsia was defined according to the criteria of the International Society on the Study of Hypertension in Pregnancy (ISSHP)[190]. Subjects with diabetes mellitus, hypertension, cardiovascular disease and/or hyperhomocysteinemia were excluded. All participants were at least 6 months postpartum, were not breastfeeding and were not taking any medication. A written, informed consent was obtained from all participants and the study was approved by the Institutional Review Board (CMO nr. 2006/080).

Protocol

Before and after a four-week cycling training program physical fitness and hemodynamic parameters were determined. All experiments were performed in the follicular phase of the menstrual cycle to minimize hormonal influence. Measurements were performed between 7.45 and 12.00 AM, under standardized environmental conditions and after an overnight fast. Participants refrained from caffeine, alcohol, smoking and vitamin C during a 12-hour period prior to the measurements.

Physical fitness test

In order to evaluate the physical fitness of the participants, we continuously recorded oxygen uptake (VO_2, $ml \cdot min^{-1} \cdot kg^{-1)}$, heart rate (HR, bpm), respiratory exchange ratio (RER, $[CO_2]/[O_2]$ ratio) and power output ($Power_{max}$, Watt) during an incremental maximal cycling test, before and after the training period. The test started at an intensity of 10 Watt and was increased every minute by 10 Watt, until exhaustion. O_2 and CO_2 concentrations were measured using an automatic gas analyzer (Oxycon Alpha, Jaeger). An electrocardiogram was used to continuously record heart rate. Two minutes after the test, a capillary blood sample was taken from the tip of the finger to examine lactate levels.

Hemodynamic function

Before and after the training period, hemodynamic parameters were determined. After 10 minutes of rest in the supine position, arterial blood pressure and heart rate were recorded at 3-minutes intervals using a semiautomatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon Company LLC, Tampa, FL). The median value of five consecutive measurements was used to represent systolic, diastolic and mean arterial blood pressure and heart rate. Pulse pressure (PP) was calculated as the difference between systolic and diastolic blood pressure.

Cardiac output (CO, $l \cdot min^{-1}$) was measured in the left-lateral position using a validated, non-invasive, inert gas rebreathing method (Innocor, Innovision, Copenhagen)[250;251]. All participants were familiarized with the technique. Prior to each rebreathing maneuver, the rebreathing bag was filled with 1.5 liter of the test gas mixture, containing 0.5% N_2O and 0.1% SF_6. The nasal airway was blocked using a nasal clamp. A constant ventilation rate of 20/min was ensured and it was emphasized that the bag was completely emptied with each inspiration. A pulse oximeter attached to the third digit of the right hand recorded heart rate and arterial oxygen saturation (SpO_2,%)[250;251]. The rebreathing technique is based on the changes in end-tidal concentration of N_2O inspired from the rebreathing bag, which determines the pulmonary blood flow. Concentrations of N_2O are corrected for total lung volume, measured by the change in concentrations of the used insoluble gas SF_6. Because cardiac output is not necessarily equal to pulmonary blood flow, the pulmonary blood flow is corrected for the shunt flow, which is determined from the subsequently measured disappearance rate of oxygen and by using the Fick principle. Then, stroke volume (SV, ml) could be calculated as CO/HR, total peripheral vascular resistance (TPVR, $dyne \cdot s/cm^5$) as $80 \cdot MAP/CO$ and global vascular compliance (GVC; $ml \cdot mmHg^{-1}$) as SV/PP.

Plasma volume (PV, ml) was measured using the Iodine-125-labeled albumin (^{125}I-HSA) indicator dilution method[4;252]. During the measurement, the subjects were in a semi-supine position on a comfortable bed. A catheter was inserted in the left antecubital vein to inject 0.2 MBq of ^{125}I. Plasma volume was obtained by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time zero, as described elsewhere[4].

We determined venous compliance (VeC, $ml \cdot dl\ forearm\ volume^{-1} \cdot mmHg^{-1}$) from the plethysmographically derived slope of the relationship between venous volume and pressure change (Hokanson, Denmark). Mercury-in-silastic strain gauges were

placed around the largest girth of the right calf and forearm, which were elevated to heart level. A pressure cuff was placed around the upper right leg and arm and connected to a rapid cuff inflator (Stopler E-20, Hokanson, Denmark) to ensure rapid and accurate filling and deflating of the cuff. The test procedure was started with an occlusion pressure of 20 mmHg and subsequent cuff pressures of 40, 60 and 80 mmHg were used for at least 2, 3, 4 and 5 minutes, respectively, to achieve a stable plateau in the plethysmographic signal. The effective pressure on the venous system was estimated as 0.8 times the cuff pressure[253]. Data were recorded at a sample frequency of 100 Hz (MIDAC, Instrumentation Department, Radboud University Nijmegen, The Netherlands) and analyzed by a customized computer program (Matlab, Mathworks, Natick, MA, U.S.A.). The venous volume variation (VVV, ml/dl) was defined as the maximal relative volume increase in a limb at each chosen cuff pressure. The VVV at different (effective) cuff pressures represents the pressure-volume curve, which was used to calculate the venous compliance.

Training program

The four-week training program consisted of two supervised training sessions per week during the first two weeks and three sessions per week during the last two weeks. The training was performed on a cycle ergometer (Ergometric 818-E, Monark, Varberg, Sweden). Each training session started with a warming-up of 10 minutes at 50% of the heart rate reserve (HRR) above the resting heart rate, which was calculated using the formula:

$$HRR = HR_{max} - HR_{rest}$$

in which HR_{max} is the maximal heart rate measured during the physical fitness test and HR_{rest} is the heart rate determined at rest.

After the warming-up period, a training intensity of 60-70% of the HRR was applied for 30 minutes. Exercise intensity gradually increased throughout the training period by 2.5% of the HRR per training session. Each training session was ended with a cooling down period of 10 minutes at 50% of the HRR.

Statistical analysis

All values are represented as median (ranges), unless otherwise stated. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 14.0.

A Wilcoxon signed rank test was used to determine the effect of exercise for all variables. We calculated the coefficient of correlation using Spearman correlation analysis. The response to the physical fitness test in HR for pre- and post-training measurements was quantified by linear regression analysis. A p-value ≤ 0.05 was considered as statistically significant.

Results

We included 9 normotensive non-pregnant formerly preeclamptic women with a median age of 34 (31-37) years and a body mass index of 24 (21-30) $kg \cdot m^{-2}$. Subjects were 17 (11-75) months postpartum at the time of inclusion. All subjects completed the 4 weeks exercise training.

Physical fitness. The 4 weeks exercise training resulted in an increase in maximal oxygen uptake and maximal work load, even though maximal heart rate had decreased and maximal RER remained unchanged (Table 1). Using RER 1.0 as a reference value for the start of significant anaerobic exercise, the training program resulted in a significantly higher workload, higher oxygen consumption and longer time needed to reach the RER-value of 1.0 (Table 1). The change in heart rate during the physical fitness test, before and after the training program, is presented in Figure 1. We compared each individual regression line of the heart rate response during the physical fitness test, as measured before and after the training period by pair-wise testing. Linear regression analysis showed different regression lines of the heart rate responses during pre- and post-training measurements (regression coefficient 0.56 (0.39-0.98) versus 0.45 (0.21-0.78) respectively, p=0.02).

Hemodynamic function. Exercise training did not alter baseline blood pressure, total peripheral resistance and cardiac output, while stroke volume increased by 10% (Table 2). Resting heart rate was 7% lower after the training period (p=0.02). In addition, plasma volume rose by 8% from 2517 (2149-2927) to 2725 (2418-3211) ml (p=0.01, Figure 2). Central hemodynamic function, as indicated by the global vascular compliance, improved by 13% (p=0.05)

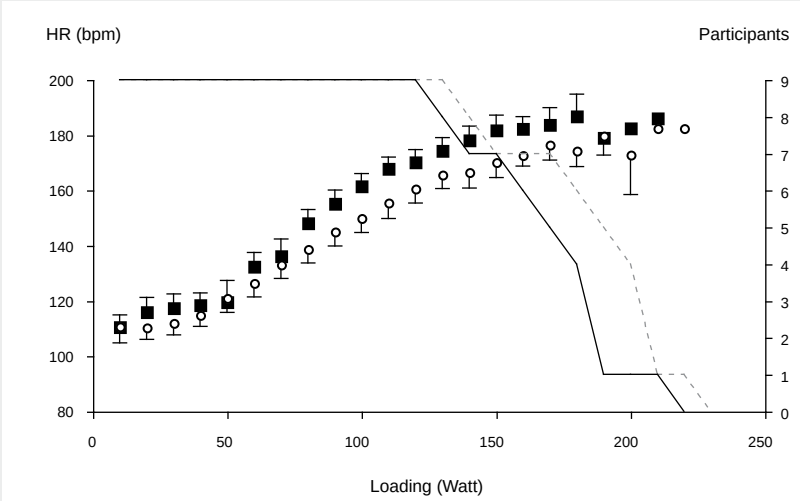
Calf venous compliance increased 18% (p=0.03) in response to the 4-week training program (Figure 3). Despite the 14% increase in forearm venous compliance from

Table 1 Physical fitness before and after the 4-weeks training protocol in 9 normotensive formerly preeclamptic women.

Parameter	Before	After	p-value
$VO_{2\,max}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	30.1 (24.5-37.1)	31.3 (21.8-38.8)	0.05
$Power_{max}$ (Watt)	170 (120-210)	190 (130-220)	0.01
Maximal RER	1.22 (1.12-1.29)	1.17 (1.08-1.43)	0.15
Maximal HR (bpm)	186 (164-212)	182 (151-204)	0.02
VO2 at RER 1.0 ($ml \cdot min^{-1} \cdot kg^{-1}$)	22.1 (15.8-25.4)	26.8 (15.0-31.0)	0.03
Power at RER 1.0 (watt)	90 (60-180)	120 (90-170)	0.02
Time to reach RER 1.0 (min)	8 (6-18)	12 (9-16)	0.02

Values are median (ranges). VO_2: lung oxygen uptake, at maximal strain ($VO_{2\,max}$), Power: workload, at maximal strain ($Power_{max}$), RER: respiratory exchange ratio, HR: heart rate.

Figure 1



The change in heart rate in response to loading during the physical fitness test, before (■) and after (○) the training protocol. The number of participants (n) who sustained the various loading levels is shown at the secondary y-axis, before (**—**) and after (—) the training protocol. Values represent mean ± SEM.

Table 2 Cardiovascular parameters before and after the 4-weeks training protocol in 9 normotensive formerly preeclamptic women.

Parameter	Before	After	Delta	p-value
Heart rate (bpm)	74 (61-86)	69 (57-79)	- 7 %	0.02
Stroke volume (ml)	72 (59-90)	79 (57-109)	+ 10 %	0.04
Cardiac Output ($l \cdot min^{-1}$)	5.2 (4.4-7.1)	5.0 (4.3-8.4)	- 4 %	0.58
MAP (mmHg)	89 (76-99)	88 (74-101)	- 1 %	0.72
Pulse pressure (mmHg)	45 (41-55)	47 (40-55)	+ 4 %	0.94
TPVR ($dyne \cdot s \cdot cm^{-5}$)	1382 (890-1580)	1316 (780-1760)	- 5 %	0.86
Global compliance ($ml \cdot mmHg^{-1}$)	1.5 (1.3-1.8)	1.7 (1.2-2.3)	+ 13 %	0.05
Red cell volume (ml)	1448 (1197-2042)	1482 (1320-2141)	+ 2 %	0.68

Values are median (ranges). MAP: mean arterial pressure, TPVR: total peripheral vascular resistance

0.042 (0.031-0.061) to 0.048 (0.023-0.089) $ml \cdot dl^{-1} \cdot mmHg^{-1}$, this change did not reach statistical significance (p=0.09, Figure 3).

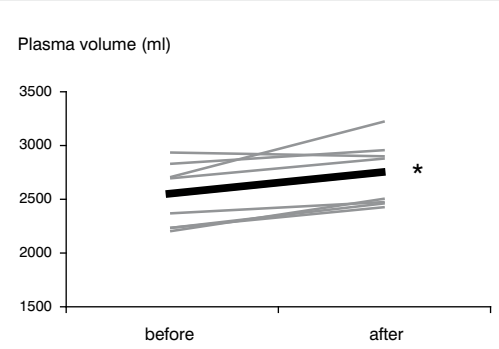
Discussion

We hypothesized that hemodynamic function, primarily venous vascular dynamics, improves after 4 weeks of cycle training in formerly preeclamptic women. We observed an exercise-mediated increase in plasma volume and calf venous compliance while resting stroke volume was higher and resting heart rate was lower at the end of the training period. These findings suggest adaptations of the venous system, but also of the central hemodynamic function, in response to an elevated level of physical activity in formerly preeclamptic women. The rise in global compliance indicates a general improvement of vascular function. The adaptations in venous compliance and plasma volume are of special interest, given the link between these parameters and an increased risk on recurrent gestational hypertensive disease in formerly preeclamptic women.

Earlier reports indicated that exercise induces changes in cardiovascular parameters in healthy individuals[133;240;243;245;254-256]. Our study group consisted of parous women with a vascular complicated obstetric history. This group of women is reported to exhibit latent hemodynamic abnormalities consistent with the early stages of chronic hypertension, like low plasma volume, low venous compliance and higher sympathetic tone[4;27;210]. The increase in plasma volume and venous compliance in response to physical exercise is relatively high in our study group. It is tempting to speculate that these correcting cardiovascular effects may reduce the risk on recurrent hypertensive disease in a subsequent pregnancy and remotely the risk on cardiovascular disease in later life.

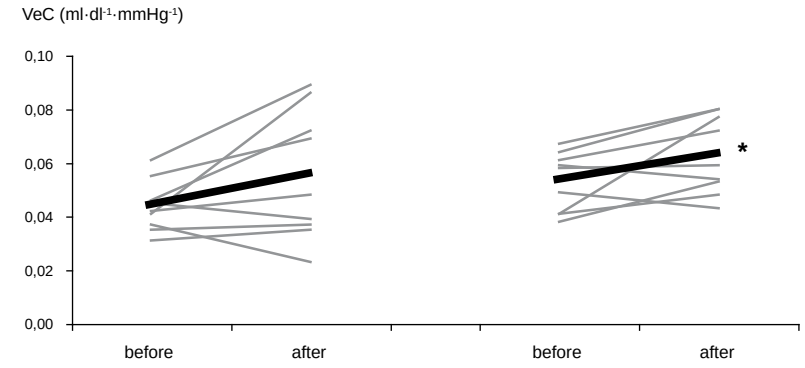
The design of our study was insufficient to study the mechanisms underlying either a primary increase in plasma volume or a primary decrease in sympathetic activity. The initial increase in plasma volume is thought to be the result of acute shifts in plasma protein content, inducing an osmotic effect with water binding. We assume that this initial increase in plasma volume results in a decrease in sympathetic activity, which improves venous compliance. Consequently, the increased venous compliance will allow buffering of the increased plasma volume in the venous

Figure 2



Plasma volume before and after the 4-weeks training program in 9 normotensive formerly preeclamptic women (thin lines). Thick line represents mean values. * $p<0.05$ compared to start value.

Figure 3



Venous compliance (VeC) of the forearm (left) and calf (right) before and after the 4-weeks training protocol in 9 normotensive formerly preeclamptic women (thin lines). Thick line represents mean values. * $p<0.05$ compared to start value.

compartment, resulting in sustained lower sympathetic activity after training in this group of women[206;207;254]. Physical activity is thought to reduce sympathetic tone by reducing baroreflex-mediated sympathoexcitation, leaving baroreflex sensitivity unaltered but reducing its activational action on the rostral ventro-lateral medulla[206]. Although we did not assess sympathetic activity, the decrease in resting heart rate during the physical fitness test supports this view[257]. Alternatively, the decrease in heart rate may also originate from the ventricular hypertrophic response to increased cardiac preload and to the rise in stroke volume[207;255]. The training-induced hypervolemia may also contribute to this bradycardiac effect of training. A 6 weeks training protocol is reported to produce substantial lowering of resting heart rate, without structural cardiac hypertrophic changes[207;258], leaving heart rate primarily modulated by autonomic outflow after short-term training protocols. The improved heart rate response after the training period also indicates adaptation of the sympathetic system.

Therefore, the observed changes most likely result from multifactorial changes in endothelial function, vasculoelastic properties and autonomic control of the circulation.

Under physiologic conditions, most of the blood volume is located in the venous compartment, where it serves as a readily available buffer to raise venous return in response to higher demands for systemic blood flow. In pregnancy, the rise in venous compliance enables accommodation of the expanding plasma volume without leading directly to circulatory overfill. In order to create an adequate blood supply for the growing conceptus, the development of a substantial amount of unstressed volume is necessary to meet the uterine demands of advanced pregnancy. In women with pre-pregnant low plasma volume, venous compliance is decreased resulting in a blunted (venous) adaptation to early pregnancy[10;11;259]. Our data indicate that even 4 weeks of moderate exercise in formerly preeclamptic women induces consistent improvement of venous compliance and plasma volume. However, one should realize that the splanchnic veins contains most of the venous blood. Active distribution of venous volume, by reducing venous compliance in the remaining part of the venous system, accounts for only 25% of the total blood transfer. We did not study the splanchnic venous response in this study, due to methodological factors, but it might be a worthwhile aspect to study in formerly preeclamptic women with low plasma volume.

Venous compliance and plasma volume are potent pre-pregnancy marker for recurrent gestational hypertensive disease[8]. Rudra and Sorenson et al. demonstrated that physical activity before and during pregnancy is associated with a reduced risk of preeclampsia[247;248]. Accordingly, our novel findings in formerly preeclamptic women may suggest that improvement of the venous vascular function by physical activity prior to pregnancy could play an important role in healthy circulatory adaptation to pregnancy and the prevention of gestational hypertensive disease in advanced gestation.

We included a heterogeneous group of nine women with a history of preeclampsia. One may hypothesize that variation in BMI may influence our results. As all women served as their own control in the comparison, it is unlikely that variation in BMI affected our observations. In addition, the variation in time since the last pregnancy may have influenced our results[23]. Correlation analysis showed that the duration of the postpartum period did not relate to any of the measured variables. Therefore, we assume that the observed changes in hemodynamic function are mediated by the training protocol, irrespective of subject characteristics.

The training program was of only moderate intensity and duration. Nonetheless, maximal oxygen uptake increased and the higher oxygen consumption and higher workload at RER 1.0 suggests that physical fitness improved after the 4 weeks cycle training. Even a short duration training protocol seems to be sufficient to induce an improvement of the vascular function and cardiovascular reserve capacity amongst women at risk for vascular complications. A longer duration may lead to more profound changes.

In conclusion, a 4-week cycling training program improves total plasma volume and calf venous compliance in normotensive formerly preeclamptic women. When low, both vascular characteristics are pre-pregnancy risk indicators of recurrent gestational hypertensive disease. We speculate that moderate intensity exercise might reduce recurrence rates of gestational hypertensive disease.

Acknowledgements

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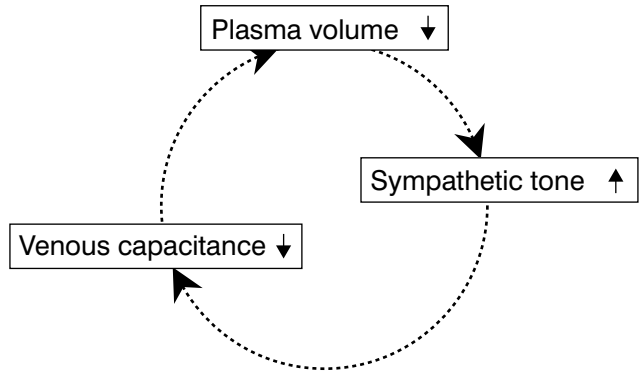
Chapter 9

General Discussion

Normal maternal vascular adaptation in the first trimester of pregnancy includes adequate plasma volume expansion to meet the increased arterial demands of advanced pregnancy. It is generally accepted that gestational hypertensive disease is preceded by blunted adaptations of the maternal vascular system in early pregnancy. Women with pre-pregnant low plasma volume are at increased risk for recurrent gestational hypertensive disease and display altered early gestational vascular adaptational changes as compared to their normal plasma volume counterparts[10;11;30]. Remarkably, in these high-risk women, the first trimester arterial response to the sudden drop in total peripheral vascular resistance, including the drop in blood pressure and a rise in heart rate, cardiac output, and stimulation of the renin-angiontensin-aldosterone system (RAAS), is non-affected[10;11]. In contrast, those women exhibit a blunted increase in venous compliance and a more pronounced rise in α-atrial natriuretic peptide (α-ANP), which in turn hampers further plasma volume expansion. Apparently, adequate functioning of the venous system is at least as that of the arterial system in healthy maternal vascular adaptation to pregnancy.

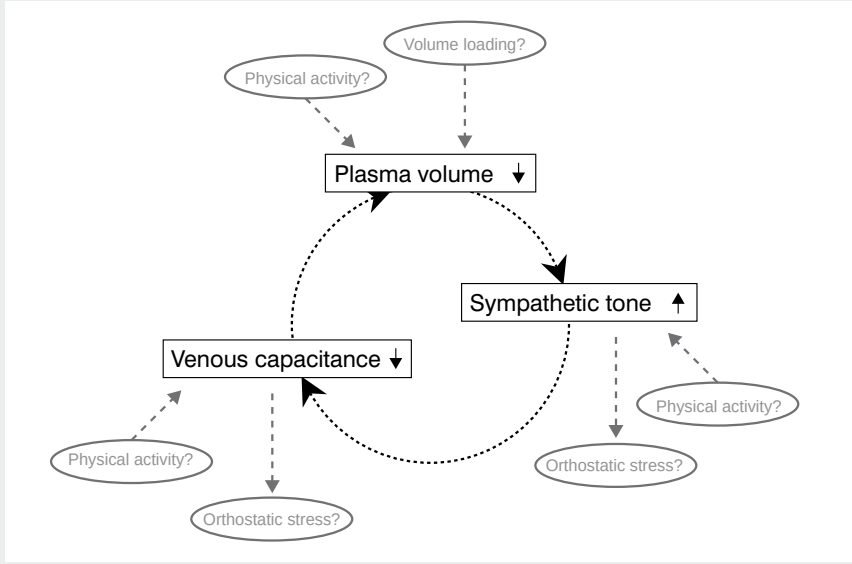
Figure 1



Simplified scheme detailing the relation between plasma volume, sympathetic tone and venous capacitance in formerly preeclamptic women.

In general, the venous system plays an important role in balancing fluctuations in arterial needs. In contrast to arteries, veins have thinner walls, higher compliance, and valves to prevent retrograde flow[132]. The main functions of the venous system are to return blood from the periphery back to the heart and to serve as a readily available buffer to maintain filling of the heart in various situations. Only small changes in venous tone and storage capacity have large effects on venous return and cardiac output. The venous reserve capacity reflects the ability to mobilize (unstressed) volume. Women with gestational hypertensive disease often have low plasma volume. Remarkably, it remains low in approximately half of patients after pregnancy. As the venous compartment contains most of the total blood volume, we proposed that formerly preeclamptic women with low plasma volume have a reduced venous reserve capacity. We studied their regulation of the venous and autonomic system, in order to better understand what factors may contribute to abnormal adaptation to pregnancy and the increased chance to develop recurrent gestational hypertensive disease.

Figure 2



Studies on the different aspects of the relationship plasma volume-sympathetic tone-venous capacitance in formerly preeclamptic women, as described in this thesis.

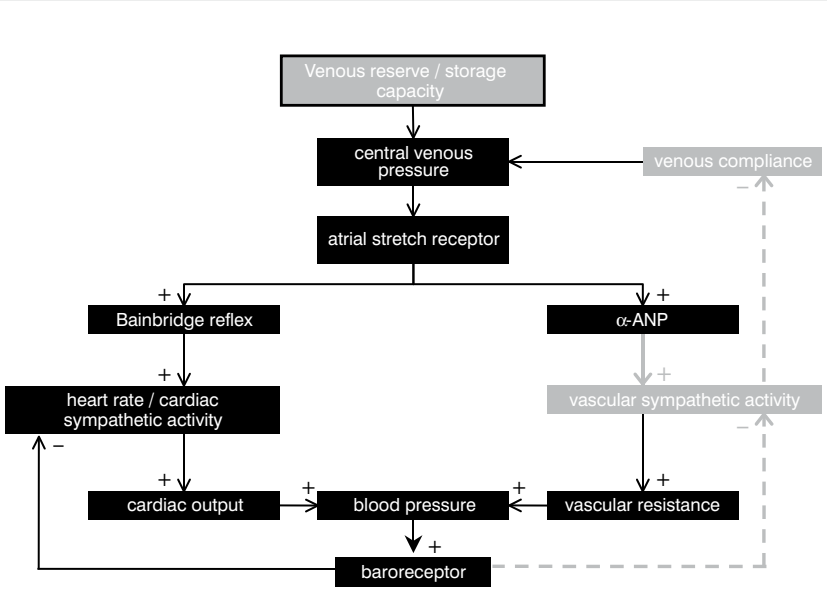
Previous studies have suggested that low plasma volume is related to a higher sympathetic tone and lower venous capacitance in formerly preeclamptic women with low plasma volume, as shown in Figure 1. The studies described in this thesis considered various aspects of this supposed relationship (Figure 2). First, we developed, modified, and evaluated methods to rapidly assess venous and autonomic function (Chapters 3 and 4). Though a range of markers is available to describe various or overlapping venous characteristics, such as venous capacitance, venous capacity, venous compliance, venous emptying rate, venous distensibility, venous volume variation, and unstressed volume[132], these are not used systematically and therefore are not easily comparable using reported data. A method to evaluate the in-vivo size of the venous compartment is currently not available. Aspects of venous functioning can be studied during orthostatic stress, when venous adaptations are needed to counterbalance the impact of orthostasis on preload. The response pattern during graded head-up tilt reflects both characteristics of the venous reserve capacity, but also concomitant regulatory autonomic changes. Therefore, we studied the reproducibility of the venous and autonomic responses at various degrees of rotation in young, healthy women. We demonstrated a reproducible reduction in venous compliance and unstressed volume and a rise in venous emptying rate during head-up tilt. One should realize that the splanchnic veins are thought to play the most important role in restoring venous return, by passive volume distribution. Active distribution of venous volume, by reducing venous compliance in the remaining part of the venous system, accounts for only 25% of the total blood transfer[260]. We did not study the splanchnic venous response in this thesis, due to methodological factors, but it might be a worthwhile aspect to study in formerly preeclamptic women with low plasma volume.

Spectral analysis is a useful, non-invasive and validated method to measure autonomic function. The autonomic response pattern to stepwise inflicted head-up tilt enables the assessment of the sensitivity and reactivity of the autonomic system, but can only be considered methodologically useful when reproducibility is at least acceptable. Autonomic function analysis may be affected by the period of hemodynamic instability directly after postural change, which reflects the time-interval needed to create a new steady state. We demonstrated that within 1 minute after discrete rotation, hemodynamic stability was reached in more than half of the measurements. Autonomic function results, assessed in 5-minute periods,

were unaffected by the time-interval after postural change, even when the first minute was included. Heart rate, blood pressure, and autonomic function have low variations between assessments during head-up tilt, but large variations when applied cross-sectionally to various subsets of studied subjects. Both studies demonstrated that head-up tilt is a useful and methodologically valid test to measure venous and autonomic functioning, especially in a longitudinal study design.

We used the developed orthostatic stress testing technique to study the assumed reduced venous reserve capacity in formerly preeclamptic women with low plasma volume in Chapters 5 and 6. In contrast to testing the capacity to raise venous return, the venous storage capacity was studied by acute volume loading in Chapter 7. In Chapter 8, the venous effects of moderate-intensity exercise of short duration in this group of women were demonstrated (Figure 2).

Figure 3



Simplified diagram of the hemodynamic and autonomic control in women with reduced venous reserve capacity.

We take the observations of the studies as described in this thesis as a proof that formerly preeclamptic women with low plasma volume exhibit a reduced venous reserve capacity along with signs of autonomic dysfunction. The results can be studied using a simplified diagram, as shown in Figure 3. In case of volume loading, the extra volume can normally be well accommodated in the venous compartment, and there will be only a minor increase in central venous pressure and atrial stretch. When activated, the Bainbridge reflex will stimulate cardiac sympathetic activity and cardiac output. The rise in blood pressure stimulates the baroreceptor-mediated inhibition of both cardiac and vascular sympathetic activity. So, in healthy venous circumstances, extra volume leads to sympatho-inhibition. Remarkably, in the low plasma volume subgroup, we observed blunted inhibition of vascular sympathetic activity. Obviously, the extra volume load could not be accommodated in the venous compartment, giving rise to a state of relative venous overfill, with a rise in central venous pressure and activation of the atrial stretch receptor. The Bainbridge reflex fails to balance the increased preload resulting in a sustained α-ANP release by the stretched atria. The absent sympatho-inhibitory response may result from either a resetting and/or defective baroreflex control of vascular sympathetic activity, from the effects of α-ANP, or both. At any rate, it indicates shallow venous storage capacity in women with low plasma volume.

Defective baroreflex function may also affect the response to head-up tilt. Head-up tilt induces an initial decrease in venous return and central venous pressure. In case of reduced cardiac filling, the baroreceptor reflex prevails over the Bainbridge reflex[261]. Therefore, under normal conditions, the decrease in cardiac output and blood pressure will result in both cardiac and vascular baroreceptor-mediated sympathetic activation. The rise in vascular sympathetic activity stimulates venous smooth muscle cells, resulting in a decrease in venous compliance and unstressed volume, which both contribute to the restoration of venous return (Figure 3). In our studies on head-up tilt, women with low plasma volume showed a consistently higher heart rate, blunted venous response capacity, and a flattened rise in sympathetic activity during head-up tilt. These observations might be attributable to autonomic dysfunction, with the inability to further constrict the veins at higher tilt angles. Veins are richly innervated by sympathetic nerves, which are deeply pierced into the smooth muscle cells of the venous wall, and are highly sensitive to sympathetic modulation[132]. The autonomic regulation of the venous system is

primarily dominated by the baroreceptor reflex system. It is has been shown in animal studies that a large increase in carotid sinus pressure induces a reduction in arterial pressure, peripheral vascular resistance, and an increase in unstressed volume. Infusion of adrenaline induced opposite effects concurrently[129;132;262]. We speculate that in our studies, the baroreflex control on vascular sympathetic activity and the venous system fails in women with low plasma volume. Nonetheless, we did not observe differences in cardiac autonomic balance and baroreflex sensitivity between groups. This indicates an intact baroreflex control on the heart, but leaves the possibility of an abnormality in the pathway towards vascular sympathetic activation[198]. In these autonomic considerations, one has to keep in mind that our data reflect autonomic changes based on spontaneous fluctuations in systolic blood pressure and heart rate, and therefore reflect arterial rather than venous control. Nonetheless, as these systems are coupled, indirectly, these data also reflect venous autonomic control.

Our observations can also be explained by an already activated contractile venous system, hampering further constriction. We and others observed a higher resting sympathetic tone and consistently higher heart rate during head-up tilt[5;6;197], which might chronically reduce venous dimensions and (resting) elastic properties of the venous wall. In this case, maximum venoconstriction is rapidly reached with head-up tilt. Lastly, an intrinsically small venous system, in line with the Barker hypothesis[194], might also account for our observed results.

Even after a short period of time, physical exercise induced a rise in resting venous compliance and plasma volume and a reduction in heart rate. These data suggest that training may be able to restore venous reserve capacity in women with low plasma volume, through a decrease in sympathetic activity. Whether baroreflex control on vascular sympathetic function also improves, remains to be elucidated.

We speculate that a reduced venous storage capacity is unfavorable in pregnancy. Comparable to our results of the study on volume loading, a situation of relative venous overfill may occur as a consequence of the early pregnant rise in plasma volume. We assume that in formerly preeclamptic women with reduced venous reserve capacity, plasma volume initially rises in response to increased RAAS-activity in early gestation. However, as venous compliance fails to increase, the extra

plasma volume cannot be accommodated in the most likely already small venous compartment. Under these conditions, central venous pressure will rise, and thereby atrial filling and atrial pressure. As detailed before, rapid fluctuations in preload can be balanced by activating the Bainbridge reflex, which raises heart rate and promotes atrial emptying. Thus, it reduces atrial stretch and, with it, the need for α-ANP release. However, at sustained venous preload, the Bainbridge reflex fails to balance the increased preload and α-ANP will be longer released by the stretched atria. Consequently-released α-ANP induces inhibition of the RAA-system and promotes renal sodium excretion at the expense of normal plasma volume expansion. With increasing arterial demands in advanced pregnancy, preload can only be maintained at higher venous sympathetic tone. The associated higher arterial sympathetic tone may induce increased blood flow velocity, endothelial shear and dysfunction, ultimately leading to gestational hypertensive disease.

The cause of the phenotype pre-pregnant low plasma volume, in combination with higher resting sympathetic tone and low venous capacitance, is currently unknown. These women are not chronically vascular underfilled since compensatory neuro-humoral changes such as elevated renin, angiotensin, and aldosterone levels are lacking and baseline hemodynamic values are quite similar to their normal plasma volume counterparts[4]. In addition, reports on renal hemodynamics and post-occlusive forearm blood flow suggest that low plasma volume is not supposed to be caused by defective renal or endothelial function[4;7;8;199]. Therefore, studies on this phenotype are different from those inducing low plasma volumes by head-down bed rest, diuretics, or space flight. Low plasma volume may originate from a chronically higher resting sympathetic activity, such as the metabolic syndrome. A constitual higher resting sympathetic tone may be present as a consequence of, for instance, hypertensive polymorphisms affecting vascular tone[263;264] or as a consequence of intra-uterine blunted venous development in line with the Barker hypothesis[194], since intra uterine deprivation primarily affects the splanchnic region.

A remaining low plasma volume after a vascular-complicated pregnancy may not only indicate those at increased risk to gestational hypertensive disease in a subsequent pregnancy, it may also point out those at increased risk to chronic hypertension and cardiovascular disease in later life. Formerly preeclamptic women have a higher chance to develop chronic hypertension, ischemic heart disease, and

stroke as compared to women with a uncomplicated obstetric history[112;208]. Low plasma volume and high vascular tone are both observed in the early phase of hypertension[27;146;209;210]. The results of our study on volume loading and head-up tilt suggest an abnormal baroreflex control on vascular sympathetic activity, which is consistent with the observations in persons with hypertension. Additionally, borderline hypertensive subjects exhibit abnormal circulatory and autonomic responses to physical and mental stress tests[265], which mimic our results of the studies on orthostatic stress. In both formerly preeclamptic women with low plasma volume and hypertensive subjects, sympathetic overactivity may increase peripheral venous tone, reducing plasma volume and venous capacity[27;146;209;210]. Therefore, we speculate that the combination of non-pregnant low plasma volume and high sympathetic activity in women with a history of preeclampsia may indicate those at risk to develop cardiovascular disease in later life. A study focused on the follow-up of those women will give insight into the contribution of this phenotype in the pathogenesis of cardiovascular disease in later life and might provide a clue to preventive strategies.

Our results on the improvement of plasma volume, venous compliance, and heart rate after a relatively short period of cycling exercise in women with a history of preeclampsia are promising. Exercise before and during pregnancy is known to reduce the chance of gestational hypertensive disease in advanced pregnancy[247-249]. We speculate that our results are related to a reduced sympathetic tone, which is expressed in the observed lower heart rate after training. A reduced sympathetic tone, higher plasma volume, and higher venous compliance before pregnancy might be beneficial in the early pregnant vascular adaptation. Therefore, it would be interesting to study the occurrence of recurrent gestational hypertensive disease after pre-pregnant exercise training in women with a history of preeclampsia. We expect a reduction in the incidence of gestational hypertensive disease in relation to the improvement of venous hemodynamics before pregnancy.

The venous adaptational changes in early pregnancy may predict the occurrence of gestational hypertensive disease in advanced pregnancy. Especially women with low plasma volume have an increased risk to develop recurrent gestational hypertensive disease in a subsequent pregnancy and will show altered early pregnant venous adaptational changes and most likely, compensatory sympathetic

responses. A model on the venous and autonomic adjustments, which can identify those at risk, would be helpful in the prevention and treatment of pregnant women with a history of gestational hypertensive disease and pre-pregnant low plasma volume. Early interventions, reducing sympathetic tone, may be the key to lower the risk of vascular derangements.

Finally, the incidence of obesity and metabolic syndrome will rise in the upcoming years. The metabolic syndrome has been related to sympathetic hyperactivity, which, in turn affects the availability of unstressed volume. In addition, obese subjects have reduced venous distensibility, either due to structural or functional factors, and the normal potency of insulin as a venodilator is reduced[234;235]. All these factors may contribute to reduced venous reserve capacity in women with high body mass index. Therefore, an overall increase in hypertensive complications in pregnancy can be expected. Exercise and weight reduction will become more and more important in studies on the prevention of gestational hypertensive disease.

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Summary

Chapter 1

This chapter is a general introduction on the studies described in this thesis. Formerly preeclamptic women with pre-pregnant low plasma volume have a three-fold higher chance to develop recurrent gestational hypertensive disease than women with normal plasma volume. Previous studies have suggested that those women with low plasma volume have relatively low venous capacitance and high resting sympathetic tone, but exhibit no compensatory neuro-humoral regulatory changes. These changes may reflect either small size or altered function of the venous compartment, and/or impaired autonomic regulation. The aim of this thesis was to improve our understanding of the regulation of the venous and autonomic system in formerly preeclamptic women with low plasma volume as compared to those with normal plasma volume. To this end, we reviewed the literature, evaluated methods to assess venous and autonomic function, and applied these to formerly preeclamptic women with low and normal plasma volume. In addition, we tried to improve venous and autonomic function in formerly preeclamptic women by exercise training.

Chapter 2

We reviewed the literature on the venous adjustments in healthy and hypertensive pregnancy. From the review, it is evident that the venous system plays an important role in the physiology and patho-physiology of maternal vascular adaptation to pregnancy. In women who eventually develop gestational hypertensive disease, as compared to healthy pregnant women, a diminished rise occurs in venous compliance and plasma volume, along with a much more pronounced increase in α-atrial natriuretic peptide (α-ANP). We speculate that in these women a higher venous sympathetic tone is required to provide enough preload to sufficiently increase blood volume flow in pregnancy. The associated higher arterial sympathetic tone increases blood flow velocity, which induces shear stress and endothelial dysfunction. This may eventually lead to gestational hypertensive disease.

Chapter 3

In this section, we assessed the reproducibility of venous responses to graded head-up tilt in healthy young women. Positive tilt induces an initial drop in venous return that reduces cardiac output. This is counterbalanced by a reduction in hemodynamically inactive (unstressed) volume and possibly also a decrease in venous compliance. In the literature ‘venous compliance’ has been measured both

on the inflow and venous outflow side. In our study, we differentiated between venous inflow and outflow measurements, and related these observations to autonomic function. During the tilt test, we observed a reduction in venous inflow values, but an increase in venous outflow values. Reproducibility of venous inflow measurements was better during tilt than the venous outflow measurements. Unstressed volume decreased with each rotational step. The venous inflow and outflow changes during head-up tilt correlated with the changes in sympathetic activity. From these observations, we conclude that venous function can reproducibly be assessed during head-up tilt. Based on the assumption that during head up tilt compliance must decrease to restore venous return, we feel that compliance should be measured on the venous inflow side, and not on the outflow side. Measurements during venous outflow do not truly represent compliance and can be more appropriately named venous emptying rate.

Chapter 4

This chapter describes the study in which we determined the variability of the blood pressure, heart rate and calculated autonomic response patterns during graded head-up tilt in healthy, young women. Because autonomic function analysis could be affected by the time needed to create steady state after postural change, we studied the duration of hemodynamic instability and its effect on autonomic function values. The variations in heart rate, blood pressure, and autonomic function between assessments were small, in contrast to the variations between and within subjects. The time needed to reach hemodynamic stability varied markedly between subjects. The percentage of heart rate and blood pressure measurements that reached stability increased from ~50% at one minute towards ~100% at four minutes, at each rotational step. Autonomic function results, assessed in a 5-minute period, were unaffected by the time-interval after postural change, even when the first minute after rotation was included. We conclude that the hemodynamic and autonomic responsiveness during graded head-up tilt can be assessed reproducibly. Blood pressure and heart rate data recorded immediately after postural change can be used to assess autonomic function.

Chapter 5

In this section, we studied the hypothesis that in formerly preeclamptic women with low plasma volume venous reserve capacity is compromised. Sufficient venous reserve capacity is needed to compensate for the reduction in preload during head-up tilt. As venous reserve capacity cannot be measured directly, we postulated that reduced venous reserve capacity is indicated by pre-syncope and/or a reduction in stroke volume during orthostatic stress. We measured the hemodynamic and autonomic responses to graded head-up tilt in formerly preeclamptic women with low plasma volume, and compared these to those in women with normal plasma volume. We observed a higher rate of pre-syncope in women with low plasma volume. The remaining women with low plasma volume, who were able to withstand the tilt test, had a lower stroke volume, and consistently higher heart rate as compared to women with a normal plasma volume. We take these results as proof that women with low plasma volume have reduced venous reserve capacity. The consistently higher heart rate suggests that this is compensated by resetting of the autonomic system.

Chapter 6

We tested the hypothesis that the reduced venous reserve capacity is caused by blunted venous responsiveness and/or autonomic dysfunction in formerly preeclamptic women with low plasma volume. In these women, the venous compartment apparently fails to restore venous return needed to maintain adequate cardiac output during head-up tilt. Venous return can be re-established by a reduction in venous compliance through sympathetic activation. We determined the change in venous compliance and sympathetic activity during graded head-up tilt in formerly preeclamptic women. In supine position, plasma volume was linearly related to venous compliance. During head-up tilt, women with low plasma volume as compared to those with higher plasma volumes showed a smaller reduction in venous compliance and a lesser increase in sympathetic activity. Our data support the view that formerly preeclamptic women with low plasma volume have indeed a reduced venous reserve capacity. This may be caused either by small venous dimensions or a preexistently activated venous contractile system, and/or by a reduced sympathetic control over the venous circulation.

Chapter 7

The aims of this study were to determine if formerly preeclamptic women with low plasma volume exhibit diminished storage capacity of the venous compartment and whether plasma volume expansion in these women reduces their resting sympathetic

tone. To this end, we compared the hemodynamic and autonomic responses to volume expansion in formerly preeclamptic women with low plasma volume to the responses in those with normal plasma volume. In women with low plasma volume, plasma volume expansion induced an increase in cardiac output and α-ANP values, and the normal sympatho-inhibitory response to volume loading was absent. There were comparable changes in blood pressure, heart rate, and baroreflex sensitivity in both groups. The changes in cardiac output and α-ANP reflect relative venous overfill after volume infusion, and thereby indicate diminished venous storage capacity. The lack of normal decrease in sympathetic activity suggests impaired cardiovascular reflex function.

Chapter 8

In this study we tested the hypothesis if 4-weeks' exercise training in formerly preeclamptic women improves venous function, as measured by plasma volume and venous compliance. Training induced an increase in plasma volume and global and calf venous compliance and a reduction in resting heart rate. This suggests that training improves venous function through a reduction in sympathetic activity. Provided that these observations are confirmed in a larger study, exercise training could well be one of the better methods to improve maternal venous function prior to pregnancy and thereby pregnancy outcome.

Chapter 9

In this section, we summarize the results of the studies described in this thesis and put them in perspective. Our results indicate that formerly preeclamptic women with low plasma volume have a reduced venous reserve capacity and impaired autonomic regulation. We speculate that the reduced venous reserve capacity will prevent normal plasma volume expansion in early pregnancy, and that the compensatory sympathetic dominance results in endothelial dysfunction and eventually in (recurrent) gestational hypertensive disease.

Samenvatting

Hoofdstuk 1

Dit hoofdstuk is een algemene introductie op de studies zoals beschreven in dit proefschrift. Voormalig pre-eclamptische vrouwen met een gering plasma volume hebben een 3 keer hogere kans op herhaling van hoge bloeddruk in de daaropvolgende zwangerschap ten opzichte van vrouwen met dezelfde voorgeschiedenis maar met een normaal plasma volume. Uit eerdere studies is gebleken dat een laag plasma volume is geassocieerd met een lagere veneuze capacitantie en een hogere sympathische tonus, echter zonder compensatoire neuro-humorale veranderingen. Dit kan wijzen op een klein veneus compartiment of een veranderde veneuze functie, en/of een verstoorde autonome regulatie. Het doel van dit proefschrift was om de regulatie van het veneuze en autonome systeem in voormalig pre-eclamptische vrouwen met een laag plasma volume beter te begrijpen. Daartoe hebben wij een literatuuronderzoek verricht, methodes om het veneuze en autonome functioneren te meten geëvalueerd en deze toegepast op voormalig pre-eclamptische vrouwen met laag en normaal plasma volume. Daarnaast hebben wij geprobeerd om in deze groep vrouwen de veneuze en autonome functie te verbeteren door middel van fysieke activiteit.

Hoofdstuk 2

Dit hoofdstuk bevat een overzicht van de huidige literatuur over de veneuze aanpassingen in de gezonde en (uiteindelijk) hypertensieve zwangerschap. Dit literatuuroverzicht laat zien dat het veneuze systeem een belangrijke rol speelt in de fysiologie en pathosfysiologie van de vasculaire adaptatie aan de zwangerschap. Vrouwen die later in de zwangerschap hypertensie ontwikkelen, vergeleken met vrouwen die een ongecompliceerde zwangerschap doormaken, vertonen een verminderde stijging in veneuze compliantie en plasma volume, in combinatie met een aanzienlijke stijging in α-atriaal natriuretisch peptide (α-ANP). Wij veronderstellen dat hiermee in deze groep vrouwen een verhoogde veneuze sympathische tonus nodig is om een toerijkende bloedtoevoer aan het hart te creëren zodat de bloedstroom voldoende kan toenemen in de zwangerschap. De gelijktijdig toegenomen arteriële sympathische activiteit verhoogt echter de bloedstroom snelheid, wat leidt schade aan het endotheel en endotheliale dysfunctie. Dit resulteert uiteindelijk in hoge bloeddruk in de zwangerschap.

Hoofdstuk 3

In dit hoofdstuk bepaalden wij de veneuze respons tijdens graduele houdingsverandering. Tijdens houdingsverandering, waarbij het hoofd ten opzichte van de voeten omhoog wordt bewogen, treedt een initiële daling van de veneuze terugvloed op, waardoor het hartminuutvolume daalt. Deze kan worden gecompenseerd door een daling van het niet actief aan de bloedsomloop deelnemende (unstressed) volume en mogelijk ook door een daling in veneuze compliantie. In andere studies wordt veneuze compliantie gemeten zowel tijdens veneuze toevloed als veneuze afvloed. In deze studie differentieerden wij tussen beide metingen and relateerden de gemeten waarden aan de sympathische activiteit. De waarden gemeten tijdens veneuze toevloed lieten een geleidelijke daling zien, terwijl de waarden bepaald tijdens veneuze afvloed een stijging lieten zien tijdens de orthostatische stress test. De reproduceerbaarheid van de waarden tijdens veneuze toevloed was beter tijdens kanteling dan die van de waarden tijdens veneuze afvloed. Unstressed volume daalde met elke rotatiestap. De veneuze veranderingen correleerden met de verhoging van sympathische activiteit tijdens kanteling. Hieruit kan worden geconcludeerd dat veneuze functie reproduceerbaar kan worden bepaald tijdens orthostatische stress. Er van uitgaande dat veneuze compliantie moet dalen tijdens positieve houdingsverandering, menen wij dat dit gemeten moet worden tijdens veneuze toevloed, en niet tijdens veneuze afvloed. De waarden gemeten tijdens veneuze afvloed representeren dus niet feitelijk de veneuze compliantie en kunnen beter ‘veneuze ledigings snelheid’ worden genoemd.

Hoofdstuk 4

Dit hoofdstuk beschrijft de studie waarin wij de variabiliteit in de bloeddruk, hartfrequentie en (berekende) autonome reactiepatroon tijdens graduele orthostatische stress hebben bepaald in gezonde, jonge vrouwen. Aangezien de autonome functie analyse beïnvloed zou kunnen worden door het tijdsinterval direct na positieverandering waarin een nieuwe stabiele situatie wordt gecreëerd, hebben wij ook de duur van hemodynamische instabiliteit en de mogelijke invloed hiervan op de autonome functie berekeningen bestudeerd. Hartfrequentie, bloeddruk en autonome functie hadden lage variatie tussen de meetsessies, in tegenstelling tot de binnen- en tussenpersoonvariatie. Het tijdsinterval om tot hemodynamische stabiliteit te komen varieerde aanzienlijk tussen de deelneemsters. Het percentage van de hartfrequentie- en bloeddrukmetingen die stabiel konden worden genoemd steeg van ~50% binnen 1 minuut tot ~100% binnen 4 minuten, bij elke rotatiestap. De autonome functie berekeningen, bepaald in periodes van 5 minuten, werden niet beïnvloed door deze periode van instabiliteit, zelfs als de eerste minuut na rotatie hierin was meegenomen. Hieruit kan worden geconcludeerd dat het hemodynamische en autonome reactiepatroon reproduceerbaar kan worden gemeten tijdens graduele houdingsverandering. Autonome functie analyse kan worden toegepast op de bloeddruk en hartfrequentie data, gemeten direct na kanteling.

Hoofdstuk 5

In deze studie toetsten wij de hypothese dat voormalig pre-eclamptische vrouwen met laag plasma volume over een verminderde veneuze reserve capaciteit beschikken. Een voldoende veneuze reserve capaciteit is nodig om de verminderde veneuze aanvoer van bloed aan het hart die optreedt na kanteling, te compenseren. Aangezien de veneuze reserve capaciteit niet direct kan worden gemeten, beschouwden wij het optreden van pre-syncope en/of een reductie in slagvolume tijdens orthostatische stress als een maat voor een verminderde veneuze reserve capaciteit. Wij bepaalden de hemodynamische en autonome reactie op (passieve) houdingsverandering in voormalig pre-eclamptische vrouwen met laag plasma volume en vergeleken deze met die van vrouwen met een normaal plasma volume. De vrouwen met laag plasma volume vertoonden een hoger percentage pre-syncope. Het resterende deel van de vrouwen met een laag plasma volume die de orthostatische stress kon weerstaan, had een lager slagvolume en een consistent hogere hartfrequentie in vergelijking met de vrouwen met een normaal plasma volume. Onzes inziens, vormen deze resultaten het bewijs dat vrouwen met laag plasma volume een verminderde veneuze reserve capaciteit bezitten. De consistent hogere hartfrequentie suggereert dat dit wordt gecompenseerd door een herijking van het autonome systeem.

Hoofdstuk 6

Wij testten de hypothese dat de verminderde veneuze reserve capaciteit wordt veroorzaakt door een beperkte veneuze respons capaciteit en/of autonome dysfunctie in voormalig pre-eclamptische vrouwen met laag plasma volume. In deze vrouwen is het veneuze compartiment blijkbaar onvoldoende in staat is tot herstel van de veneuze terugvloed aan het hart, om een adequaat hartminuutvolume te waarborgen. De reductie in veneuze terugvloed kan worden gecorrigeerd door

een daling in veneuze compliantie, door middel van sympathische activatie. Wij bepaalden de verandering in veneuze compliantie en sympathische activiteit tijdens graduele orthostatische stress in voormalig pre-eclamptische vrouwen. Plasma volume correleerde lineair met de veneuze compliantie in horizontale positie. Tijdens kanteling vertoonden vrouwen met een laag plasma volume, vergeleken met vrouwen met hogere plasma volumes, een beperkte daling in veneuze compliantie en een mindere stijging in sympathische activiteit. Onze data ondersteunen de veronderstelling dat vrouwen met een laag plasma volume inderdaad een verminderde veneuze reserve capaciteit hebben. Dit kan worden veroorzaakt door een klein veneus compartiment of een reeds gecontraheerd veneus systeem, beide zonder het vermogen tot verdere venoconstrictie tijdens orthostatische stress door gestoorde autonome regulatie.

Hoofdstuk 7

Het doel van deze studie was om te bepalen of voormalig pre-eclamptische vrouwen met laag plasma volume een verminderde buffer capaciteit van het veneuze compartiment hebben en of plasma volume expansie in deze vrouwen de sympathische tonus kan laten dalen. Daar toe vergeleken wij de hemodynamische en autonome reacties op volume expansie in voormalig pre-eclamptische vrouwen met laag plasma volume met die van vrouwen met een normaal plasma volume. In de vrouwen met laag plasma volume induceerde de plasma volume expansie een stijging in hartminuutvolume en α-ANP, en was er geen (normale) daling in sympathische activiteit. Er waren vergelijkbare veranderingen in bloeddruk, hartfrequentie en baroreflex gevoeligheid in beide groepen. De veranderingen in hartminuutvolume en α-ANP suggereren een relatieve veneuze overvulling dat een verminderde veneuze buffer capaciteit weergeeft. De afwezige daling in sympathische activiteit impliceert een verminderde cardiovasculaire reflexfunctie.

Hoofdstuk 8

In dit hoofdstuk testten wij de hypothese of een 4-weekse fietstraining in voormalig pre-eclamptische vrouwen de veneuze vaatfunctie verbetert, bepaald door plasma volume en veneuze compliantie. De training resulteerde in een stijging van het plasma volume en veneuze compliantie, zowel globaal als aan het been, en een daling in rust hartfrequentie. Deze data suggereren een verbetering in veneuze vaatfunctie, door een daling in sympathische activiteit. Mits deze bevindingen worden bevestigd in een grotere studie, kan er worden geconcludeerd dat fysieke activiteit een van de betere methodes zou kunnen zijn om voor de zwangerschap de veneuze vaatfunctie te verbeteren en daarmee zwangerschapsuitkomst.

Hoofdstuk 9

In dit hoofdstuk vatten wij de resultaten van de studies, zoals beschreven in dit proefschrift, samen en plaatsen de resultaten in perspectief. Onze resultaten tonen aan dat voormalig pre-eclamptische vrouwen met laag plasma volume een verminderde veneuze reserve capaciteit hebben en een gestoorde autonome regulatie. Wij veronderstellen dat de verminderde veneuze reserve capaciteit een normale plasma volume expansie belemmert in de vroege zwangerschap, en dat de compensatoire sympathische dominantie resulteert in endotheliale dysfunctie en uiteindelijk in herhaling van hoge bloeddruk problemen in de zwangerschap.

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Het leven is mooier met jou!

Curriculum Vitae

Ineke Krabbendam werd op 18 september 1978 geboren in Apeldoorn. Op 4-jarige leeftijd verhuisde zij met haar ouders en broers Arjan en Wilfred naar het Twentse Goor. In 1996 slaagde ze voor het VWO aan de Scholengemeeschap de Waerdenborch, te Holten. In datzelfde jaar verhuisde ze naar de Domstad om te starten met de studie Geneeskunde.

Aan het eind van de doctoraalfase werd als wetenschappelijke stage een studie gedaan naar de relatie tussen thrombofilieën en herhaalde miskramen in Adelaide, Australië (prof. G.A. Dekker, University of Adelaide). Het keuze co-schap (obstetrie) werd besteed aan een het schrijven van een kritisch review over hetzelfde onderwerp (dr. A. Franx, UMCU). Ook deed zij 2 co-schappen in Maleisië en (wederom) Australië.

Na het afronden van de studie geneeskunde heeft zij eerst met veel plezier als ANIOS gewerkt in de Isala Klinieken, te Zwolle (opleider dr. H.H. de Haan). In 2005 begon ze als arts-onderzoeker Verloskunde aan het UMC St. Radboud, te Nijmegen. Het onderzoek naar de veneuze reserve capaciteit en autonome regulatie bij vrouwen met een voorgeschiedenis van pre-eclampsie heeft geresulteerd in dit proefschrift.

In januari 2009 is zij gestart met de opleiding Obstetrie & Gynaecologie binnen het cluster Nijmegen.

Ineke Krabbendam is getrouwd met Andre Pool en in 2007 kregen zij samen hun zoon Douwe Joris.